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Predictive biomarkers in the treatment of colorectal cancers

L. Landherr

Standard treatment of advanced colorectal cancer (CRC) contains some kind of fluoropyrimidine, oxaliplatin or irinotecan. Thanks to the supplementation of chemotherapy with targeted biological agents (bevacizumab, cetuximab, panitumumab), results improved further and today survival exceeds 2 years even in the case of advanced tumors. Moreover, hepatic metastases may become operable after effective therapy, thus certain patients may recover completely. Genetic characteristics of tumors significantly affect both response to treatment and toxicity, therefore, on the basis of such characteristics, it is possible to select those patients that will respond differently to therapy. Thus toxicity associated with unnecessary (and very expensive) treatment can be avoided and patients benefiting the most from therapy can be selected.

In this publication, the author provides a review of biomarkers related to drugs involved in CRC treatment and influencing the activity thereof.[2]

Key words: biomarkers, KRAS, Microsatellite instability, prediction

Highly complicated activity of the intracellular signal transduction pathways is necessary to the uninhibited progression of tumor cells. Thanks to the progress in molecular pathology we recognize these mechanisms increasingly better and targeted inhibition of signaling turns possible.

In our outpatient clinic 129 CRC patients were treated with targeted therapy during 2000 and 2009. Before the evaluation of their genetic appearance I would like overview the genetic background of the mostly applied drugs.

Fluoropyrimidines (5-FU)

5-FU is converted into fluorodeoxyuridine (FUDR) by thymidine phosphorilase (TP) and is further converted into the active metabolite, fluorodeoxyuridine monophosphate (FdUMP). Toxicity in the form of neutropaenia, stomatitis, diarrhoea and, in the case of prolonged treatment, hand-foot syndrome is almost exclusively caused by the inhibition of thymidylate synthase (TS).[26] Inhibition of TS leads to a decreased production of deoxythymidine mono-phosphate (dTMP), reducing both the synthesis and the repair of DNA. In the case of amplification/overexpression of the TS gene, 5-FU is ineffective. However, when the levels of its metabolizing enzyme, TP are low, an increased effect can be expected.[18]

Polymorphism in the promoter region of the TS gene explains differences in TS expression (and the variable sensitivity to 5-FU), but efficacy and toxicity is also significantly influenced by the amount of the enzyme (dihydropyrimidine dehydrogenase=DPD) involved in the degradation of 5-FU prior to its elimination. In the case of tumors with low DPD expression, a higher efficacy of 5-FU can be expected, higher exposition and lower clearance in turn may lead to severe gastro-intestinal bleeding and haematological toxicity. Consequently, a higher tumor response to fluoropyrimidine treatment can be expected with lower TS and DPD levels.

Irinotecan

Irinotecan acts through interaction with the topoisomerase 1 (TOPO1) enzyme: irinotecan stabilizes temporary single-strand DNA breaks caused by TOPO1, DNA becomes fragmented and the cell dies. The drug is eliminated from the body via UDP-glucuronosyltransferase (UGT1a1) after its conversion into an active metabolite (SN-38). Polymorphism of UGT1A1*28 leads to decreased UGT1A1 expression. Serum drug levels are elevated, toxicity is increased and the metaanalysis of 10 studies shows that the UGT1A1*28 genotype is associated with irinotecan toxicity.[11]

Oxaliplatin

Its cytotoxicity is based on the formation of cross links between DNA strands. Inhibition of the formation of cross links through the "nucleotide excision repair" (NER) signaling pathway leads to oxaliplatin resistance. Higher levels of the ERCC1 protein results in increased DNA repair and a decreased antitumor effect. The XPD gene is responsible for the helicase involved in the functioning of the signaling pathway. It would be especially important to know whether FOLFOX used in the first-choice treatment of metastatic CRC or FOLIFRI is more efficient and

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which of them results in lower toxicity. Pharmacogenetic testing of UGT1A1*28, ERCC1-118T, XPD-751C or glutathione S-transferase (GSTP1-105 G) alleles may help decide this question.[8,21]

Anti-EGFR monoclonal antibodies

Activation of EGF receptors brings a number of intracellular signaling pathways (Ras/raf/MAPK) into action, resulting in increased proliferation, angiogenesis and metastatisation and decreased apoptosis. Anti-EGFR monoclonal antibodies prevent the binding of EGF (the ligand) to the receptor. As regards CRC, two medicinal products are currently marketed: cetuximab and panitumumab. The situation is even more complex because of the fact that, at one step of the intracellular signaling pathway, mutations of KRAS activate the Ras/raf/MAPK signaling pathway (in an "acquired manner"), irrespective of whether the ligand has bound to the receptor or not, thus the inhibition of EGFR becomes ineffective.[15] The mutation of KRAS is observed in almost 30% of cases. Clinical observations have proven the theory: 318 patients showing progression after irinotecan-based chemotherapy were treated with cetuximab in 5 studies and response was seen in 32-64% of patients having non-mutant (wild type) KRAS, compared to 0-10% in the case of mutant KRAS and PFS decreased from 22-31 weeks to 10-12 weeks.[5,15] Italian researchers identified KRAS mutation in 30% of patients after a posteriori examining 113 metastatic colorectal cancer samples and found this to be associated with resistance to cetuximab and panitumumab treatment (p=0.11), but BRAF mutation was also considered to be a very important negative prognostic factor.[6] Where BRAF mutation was present, treatment was totally ineffective despite the wild-type KRAS and none of the responding patients showed BRAF mutation (p=0.029). Accordingly, in patients with BRAF mutation, both progression-free survival (PFS) (p=0.11) and overall survival (OS) (p<0.0001) was shorter than in wild-type patients. This implies that KRAS and BRAF are components of the same signaling pathway, in contrast with PIK3CA mutation that forms part of the PI3K-AKT signaling pathway functioning parallelly with Ras/raf/MAPK and these together may have synergistic effects on the downstream mTOR signaling pathway.[28] According to the findings of the Italian researchers, the sensitivity of tumor cells to cetuximab and panitumumab is maintained when administering BRAF-inhibiting sorafenib. Wong and Cunningham observed the same: both response rate and PFS was improved in patients carrying wild-type KRAS treated with FOLIFRI plus cetuximab, while the addition of cetuximab did not provide any benefit in patients with KRAS mutations and none of the patients with KRAS mutations responded to panitumumab.[29] After analyzing 394 CRC tumor samples, Australian and Canadian researchers found at least one mutation in exon 2 of the KRAS gene in 42.3% of the specimens. In the case of wild-type KRAS, overall survival was 9.5 months with cetuximab treatment and only 4.8 months with best supportive care (BSC) and progression-free survival was 3.7 months and 1.9 months, respectively. Patients with mutant KRAS demonstrated no benefit of cetuximab treatment in OS, nor in PFS.[13] Today we know that the lack of expression of the PTEN protein, a downstream component in the EGFR signaling pathway, also predicts inefficient treatment with cetuximab.[7]

Anti-VEGFR treatment with bevacizumab significantly improves disease outcome both in patients with wild-type and with mutant KRAS, although clearly to a greater extent in the case of the wild type. The addition of bevacizumab to IFL treatment increases PFS from 7.4 months to 13.5 months in patients wild wt-KRAS (p<0.0001), while PFS is also significantly increased in cases where m-KRAS is present (from 5.5 months to 9.3 months, p=0.0008).[12] The relationship between KRAS mutation and the inefficiency of panitumumab monotherapy is also known: out of 208 subjects, 0% of patients with mutant KRAS and 17% of wild-type patients responded to panitumumab treatment, the latter also showing longer PFS and OS.[1] In addition to KRAS and BRAF status, EGFR gene copy number also correlates with response rate: copy numbers higher than 2.47 or chromosome 7 polysomy present in more the 43% of the cells are associated with a response rate of approximately 30%, while in the case of lower values, response rate is 0% (25). Dutch researchers also found that EGFR gene copy number significantly predicts the probable response to cetuximab treatment (p=0.016) and overall survival (p=0.005), independent of the KRAS status.[22] In the BOND study, irinotecan-refractory CRC patients were treated with irinotecan plus cetuximab or cetuximab alone: the combination was efficient in 22.9% of patients and monotherapy in 10.8%.[4] It was found that the level of EGFR expression in itself does not predict probable response; indeed, some patients with no detectable EGFR expression also responded to cetuximab.[3] Concomitant use of targeted therapies does not always enhance efficacy: for example, combination of chemotherapies using bevacizumab and anti-EGFR treatments is also contradictory. In a paper of Dutch researchers on 755 patients, the addition of cetuximab to capecitabine-oxaliplatin-bevacizumab treatment resulted in a decrease of median PFS (from 10.7 months to 9.4 months), suggesting a negative interaction between bevacizumab and cetuximab.[27] Similar results were seen in the PACCE study involving 823 patients. So it is no accident that Mayer says, referring to the unnecessary anti-EGFR treatment, that "more is not always better".[19] Therefore it is recommended to test mutations of the KRAS codon 11 and 13 in all patients waiting for cetuximab or panitumumab therapy, thus it is possible to select those patients for whom very expensive cetuximab and panitumumab treatment is not expected to be efficient and unnecessary (and expensively generated) toxicity could be avoided.

Microsatellite instability

One of the driving forces of carcinogenesis is genetic instability, an example being the lack of the

repair of DNA replication errors. Due to the mutations of mismatch repair (MMR) genes, the MMR system is unable to repair such DNA errors, so mutations accumulate in short repetitive sequences ("microsatellites") – this is why we talk about microsatellite instability (MSI). MSI means that the length of short repetitive nucleotide sequences (microsatellites) in the tumor DNA has changed (is "instable") as compared to normal DNA (less frequently called a replication error or mutator phenotype). The phenotype of a tumor lacking MMR is called "high frequency microsatellite instability" (MSI-H). This is seen in 15% of CRCs.[17] Such MSI-H tumors are significantly more benign at an early stage, metastasize to regional lymph nodes or distant organs less frequently, probably need no adjuvant chemotherapy or such chemotherapy may even worsen results.[9,23] As regards advanced cases, MSI may predict the probable response to pharmacological treatments. In the Dutch DCCG study, the levels of MMR proteins were measured (MLH1, MSH2, MSH6, PMS2) and in MSI-high cases a response rate of 58%, in cases without MSI a response rate of 83% was seen with different chemotherapies (p=0.03). Overall survival was 7 months in the previous case and 18 months in the latter, therefore microsatellite instability in CRC means a reduced response to chemotherapy and thus a significantly worse prognosis.[14] It would be recommended to routinely test 5 to 10 markers would highlight the deficiencies in the mismatch repair system and the global microsatellite instability of CRC.[20]

CONCLUSION

Use of the genetic predictive markers will allow us the use of tailor made therapy in the next future.

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The way of an english for medical purposes language testing system into the EU

G. Rébék-Nagy, V. Warta

Of the language teaching trends of the 20th century the social demands for Teaching English for Medical Purposes (TEMP) has markedly delineated. Simultaneously with employing English for Medical Purposes (EMP) interpreters and translators, the need for medical professionals aware of the register, discourse and major genres of the international biomedical community tends to increase even more rapidly. The obvious explanation to this lies in the advantage that through their professional socialization these health care providers possess the kind of background knowledge that underpins a special purpose language use.

Languages for Specific Purposes (LSP) tests received a great amount of interest in Hungary in the last decade of the 20th century. Relying on the state accreditation system LSP testing systems in a number of subject areas (business, agriculture, commerce, economics, law, public service, theology, military and police affaires and medicine) have been developed in accordance with the recommendations outlined in the Common European Framework of Reference for Languages (CEFR, 2002). Of these LSP tests the present paper will discuss the EMP testing system, and also the method through which these bilingual exam papers can be made available as output requirement for medical universities in the European Union, Japan, Chine and South Korea.

For the implementation of this rather ambitious project, financial means are essential. A brainstorming session with participants from seven countries (Austria, Hungary, Poland, Romania and Spain from the EU and Japan and Serbia from outside the EU), was held in Budapest, Hungary on 6-7 January 2009 to form a consortium for the purposes of fund raising. England and France have also joined this consortium in the meantime. The purpose is to submit applications to the Council of Europe relying on the currently bilingual PROFEX EMP testing system accredited according to EU standards.

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The international demand for the new testing system is shown by the fact that the linguistic aspects of medical communication tends to gain an increasing coverage in prestigious peer- reviewed international medical journals. For instance, "Chest", a highly valued medical journal on thoracic surgery coming out in the United States, in addition to medical research articles, has developed a column entitled Medical Writing Tips dealing with medical communication. The idea of the present study was also raised in this journal (Rebek-Nagy, Warta and Barron, 2008). The acronym sTANDEMpex (standardized English for Medical Purposes Exam) coined by the members of the consortium of EMP experts is meant to explain the main features of the project. This exam is planned to be a set of internationally available skills based EMP proficiency tests. The word TANDEM hidden in the middle of the acronym refers not only to the wide range of application of the end product but also to the need for distributing and harmonizing rather complex tasks and activities leading to launching the first pilot tests.

Of the great number of important details the cultural determination of the new testing system deserves special attention. The measuring instrument meant for international utilization forced the team of developers to decide whether they should take national peculiarities and conventions of biomedical communication into account or create a new system based on global culture. As the former solution may raise a number of insurmountable difficulties in the course of standardization, the team of developers adopted the latter philosophy. In addition the inclusion of an optional module was also decided upon, which, on the other hand, is still capable of catering for at least a part of the national features of medical communication.

Apart from the cultural implications general features of the STANDEMpex EMP exam also requires urgent decision. At the Budapest meeting consensus was reached that the exam needs to be monolingual (English), will cover three skills levels in accordance with the recommendations of the CEFR (B1, B2 and C1), its task will reflect direct rather than indirect methods of language testing while the dichotomy of

testing accuracy and fluency need to be implemented with a slight dominance of fluency, although accuracy needs to be born in mind at all times during test development. In accordance with this decision was made that the proportion of discrete point and integrative test tasks should be 40:60 percent, respectively. A major testing principle was also formulated at the meeting: the target skills of the exam are language and communication skills, which are based on professional background knowledge. The degree of engaging this knowledge in solving test tasks increases with the level of the target language skills. In other words, the C1 level exam is impossible to pass without substantial biomedical background, while tasks level B1 may be solved relying on the general medical knowledge, a man in the street is supposed to have.

The exam uses tasks focusing on authentic EMP texts. The optimal solution of such tasks is possible by relying on the knowledge of the appropriate language register and genres, which are part of professional background knowledge. As this kind of knowledge does not concern the biomedical content, there is no obstacle to engage it in solving the exam tasks.

It is desirable that oral and writing skills of medical communications are proportionally represented in the exam. Wants and needs analyses previously conducted in the participating countries revealed that both national and international biomedical communication require both oral and written receptive and productive language skills.

Method of test development

The springboard for the planned testing system will be the accredited PROFEX, an already existing set of currently bilingual EMP tests aligned to CEFR skills levels.

The standardization of the series of tasks developed in accordance with the principles outlined above was carried out relying on the widely used internationally available Manual published by the Council of Europe (2003). The first stage of this procedure is the familiarization with the skill levels described in the CEFR was carried out at the test developers' meeting in Budapest, Hungary. Descriptors concerning the individual skills levels and skills, as well as the test specification were developed as a result. In addition to the above, internal validation will involve assessment, the analysis of the results, the description of the process of the test development and the critical review of the whole examination procedure. As a closing act of the specification phase the individual parts of each series of tasks will be described using descriptors specified in CEFR.

The next phase of the standardization process is the standardization of assessors' value judgments. This phase includes practising the assessment of EMP language performance in accordance with CEFR levels and also the estimation of the difficulty level of the individual test items.

The training sessions will be organized as on-line video conferences and power point presentations.

The third phase of standardization is empirical validation. The first step of this process is internal validation, which is meant to identify the psychometric quality of the tests. The second step is external validation, which uses two of the currently available three EMP testing systems (Tokyo Medical University (TMU) EMP Tests, and PROFEX administered by the University of Pécs). The external validation relying on anchor tests aims at establishing relation between sTANDEMpex and CEFR relying on independent measuring instruments.

The limited length of the present study gives an opportunity for only presenting the descriptors selected at C1 level from CEFR.

The listening comprehension skills at this level are characterized by three parameters:

-the candidate can easily follow interaction on complex and unknown topics between several participants

-the candidate can easily follow presentation, talk, argument

-the candidate can understand small details, suggested attitudes and the nature of relationship between the speakers.

Reading comprehension is described by four descriptors:

-the candidate can understand demanding, long texts

-the candidate can understand hidden meanings

-the candidate can skim long and complex texts and find the important details

-the candidate can identify the content and the importance of topics in a wide range of professional contexts.

There are also four parameters for the description of speaking skills:

-the candidate can provide detailed description of complex topics and can link the individual sub-topics, can relate the individual points and use appropriate closing

-the candidate can make announcements fluently, is able to express the finer shades of the message using appropriate accent and intonation

-the candidate can give a clear, well structured presentation on complex topics

-the candidate can easily handle interruptions.

Writing skills are also described by three descriptors:

-the candidate can create well-structured written texts on complex topics

-the candidate can reliably use cohesion devices

-the candidate can relate complex topics in a well-structured manner

-the candidate can use additional ideas, arguments and appropriate examples for relating and supporting his views.

In the optional mediation module the requirement is translation from the candidate's national language into EMP:

-the candidate can mediate between his first language and the target language in genres presupposing complex professional communication skills

-the candidate can produce well-structured and detailed texts on complex topics

-the candidate can reliably use cohesion devices.

As the above mentioned descriptors indicate the selection was focused on parameters describing general language performances but these parameters can always be related to language performance required in professional communication. This principle is referred to in the individual descriptors.

The first steps towards sTANDEMpex an international system of EMP language tests have been taken, the familiarization with CEFR, the descriptors identifying the individual levels and skills and the alternatives of the exam specification have been completed.

A further task is the finalization of one of the alternatives. This first of all means the assessment system of the exam, i.e. the decision whether the assessment should take place at each level or calibrated band descriptors should be used.

A further step is to set up a team of assessors whose members need to be trained. The last phase of standardization is the most time-consuming and demanding internal and external validation which are hoped to be carried out using the resources of an EU grant.

The finalized set of EMP tests will be made available in and outside the EU, with special regard to Japan, China and South Korea and Serbia in order that medical universities and faculties in these countries can use it at their own discretion as a professional language output. This step may lead to the standardization of the communication within the international biomedical discourse community as well as the doctor-patient interaction.

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A corpus linguistic study of medical english use in drug information websites

Alexandra Csongor

It is worth using opportunities offered by the Internet both in teaching and learning Medical English. It is also a challenging and new field of study as Internet linguistics emerged in about the last 20 years. The Internet as a new medium has had an impact on language use.[3] Computer mediated communication differs considerably from traditional writing, therefore the analysis of drug information websites particularly focuses on elements of written and spoken language. The study also examines the visual elements and the content of the randomly selected texts. It also aims to investigate the communicative function of the information given about a particular prenatal vitamin. The main focus is on linguistic elements that characterise the special communicative style of Internet texts, for example sentence structure, modality, the presence of passive structure and nominalisation are examined.
Key words: Medical English, Internet texts, drug information

The use of the Internet has induced and is continously generating new forms of language use. The present study aims at analysing the influence of a new communication medium on Medical English. The goal of the study is to present a special area of Medical English use with the analysis of 14 drug information websites of prenatal vitamins.[4-17] After identifying the communicative functions found in this genre the linguistic forms realising these functions are analysed Although some linguists perceive the effect of new technology as a deterioration of written communication the author's presumption is that websites became less formal and represent a creative and dynamic new style in this type of communication.[3] Some characteristics of spoken and written language in the randomly selected texts will be presented.

MATERIAL AND METHOD

It is a sociolinguistic study in terms of approaching language as a social activity. It focuses on specific patterns and rules of typical websites containing drug information. We can examine for example, how different informative, persuasive or consultative techniques get manifested in language forms.

The special language use of Internet texts, which seems to be closer to an informal stylistic variety, was analysed with corpus-based techniques.

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The basis for analysis is the corpus consisting of 14 English websites containing drug information about prenatal vitamins. The websites were selected by different search programmes on the Internet, all include drug information about prenatal vitamins[4-17] and are informative rather than scientific in character. The information provided aims at patients who take or are interested in these medications. Various levels of discourse such as sentence structure and syntactic structures like passive, nominalisation or modality are studied. These linguistic features allow to compare the Internet texts to traditional written communication.

RESULTS AND DISCUSSION

One part of the analysed web pages contain a lot of visual elements, however, it is a common feature of all texts in this communicative medium.[9,10,11,14] One reason for using pictures, photos etc. is probably to arouse interest in the reader. A number of hyperlinks can be found in connection with the topic, still the reader can easily navigate through the information. The structure of the pages is manageable, the texts consist of 2 or 3 columns, the main column carrying the most important information added by other informative links or commercials.

The discourse structure in these documents is composed of simple sentences, which describe the medication. Instructions are given in short imperative sentences e.g.:

"Take one tablet daily."[11]
"Swallow with a full glass of water."[14]
"Take 2 capsules twice a day."[10]

The lack of nominalisation gives an informal tone to the discourse, the writings are more like conversation. It is also supported by a lot of word repetition used by the authors. There is a number of other features that are characteristic of spoken communication. They rarely use passive voice, complex sentences or relative clauses.[2] As opposed to the standard subject –predicate form, the following incomplete structures are frequently used:

"Ideal for sensitive, pregnant stomach."[11]
"Helps tissue and skin development."[11]

The metalinguistic elements often used in writing such as besides, moreover, however, in spite of etc. are avoided in the documents studied. However, traditional written features also appear. Information is often concentrated around one reference point in a sentence, such as "small, easy –to swallow, once- daily formula:" In most cases the discourse is made up of complete sentences, contractions are not often used.[2]

Regarding their content the texts provide information about the components of the particular vitamin, instructions and precautions in all cases. Drug information sites aim to involve all necesarry information and use repetition and simple linguistic structures.

One reason for this can be to avoid later complaints in connection with the medication. A warning to see a health care professional at the bottom of the documents usually appears. In this typical part of the drug information texts the main communicative function is commercial persuasion via Internet.

In another category the web documents represent a more formal and scientific style, and are content-based rather than commercial websites.[4,5,6,7,8,12,13,15,16,17] The discourse is characterised by the common use of passive and other elements of academic writing appear such as nominalisation eg.:

"The tablets are given..."[4]
"They may also be prescribed to improve..."[5]
"Nutritional supplementation is especially important during pregnancy."[4]

However, the discourse is close to traditional written communication, elements of spoken language use can be found. Compound sentences are rare, the same syntactic structures are repeated.[2]

"These products contain..."[5]
"Take these products..."[4]
"Tell your doctor..."[6]
"Tell your pharmacist..."[6]
"How should you use..."[6]
"How to take it..."[6]

A personal tone is achieved with the use of modal auxiliaries and the personal pronouns I and you, like in you should, what should I avoid etc.

Less visual elements are present, there is more focus on the content. All necesarry information is provided for the reader such as components, instructions and precautions. Date and reference can be found at the bottom of the page in a number of cases. [3,6,12,13,14,15] There are explanations added to the data given in more cases than in commercial websites. According to Beaugrande didactic texts convey information and facts about an established topic.[1] To sum up, the communicative function of these Internet documents is to instruct the reader by providing adequate information. They are usually found on home pages of medical institutions or medicine databases.

CONCLUSIONS

The texts appearing on the Internet differ in fundamental respects from traditional writing. First of all, they are more dynamic and closer to speech in character, which means that a text can be refreshed or modified at any time. An author is able to react to the readers' opinion, question, request while a printed text cannot be changed. Another important communicative capability of websites is hypertextuality.[3] A page provides a network of links to a number of other texts on the Internet.

Finally, the language use of the analysed websites confirm the hypothesis that in Internet texts informal elements are more common than in traditional written language.

The above examples illustrated that the analysed website texts in the field of Medical English represent both written and spoken linguistic features. The language use of the Internet considerably affects the communicative style of the authors, which is more creative and alive than printed writing but at the same time more regulated than speech.

Researchers may find it to be useful to exploit the linguistic potentials offered by computer mediated communication both as a reader and as an author.

The extension of the corpus with other fields of Medical English is necesarry for a thorough analysis, which can yield a large amount of data, thus providing

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5 http://www.drugs.com/pdr/materna.html

6 http://medsafe.govt.nz/Consumers/cmi/e/Elevit.htm
7 http://www.pregnacare.com/uk/plus_fom.aspx
8 http://pharmabook.net/en/metaboliki/makro—i-mikroelementy/pregnavit.html
9 http://www.amazon.com/o/ASIN/B000TFN8SW/105-8123110-7218803?SubscriptionId=14H876SFAKFS0EHBYQ02
10 http://www.herbalremedies.com/45130.html
11 http://www.pregnancy-plus.com/supplementfacts.html
12 http://www.talkmedical.com/medications/4437/Obegyn
13 http://www.drugs.com/pdr/natachew-tablets.html
14 http://www.healthdigest.org/prescription-drug-reference/natafort-oral-8277.htm
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Some communication problems in the forensic medical discourse community

Katalin Fogarasi

Following accidents and injuries a specific communication will take place between physicians and public health officers or coroners, in which the clinician is supposed to describe the location, the extent and type of injuries with the greatest possible accuracy. This is a particular discourse community, whose genre, the diagnostic findings, is regulated by strict rules concerning format and language use. The public health officer or coroner is to report to the authority (police, court, insurance, etc.) on the quality of the injury based on this document when it is properly completed.

However, in Hungary the terminology is not standardized, as a consequence the description of the injury in Hungarian, the diagnosis in Latin and its obligatory Hungarian translation often shows disturbing differences in content. In a number of cases even a re-examination of the patient is required. On the basis of authentic diagnostic findings, the present study aims to investigate how far the problems of the non-standardized terminology system affect successful communication of this specific discourse community and offers a possible solution to the problem caused by the lack of standardization.

Key words: discourse analysis, medical terminology, genre analysis, corpus linguistics

Following accidents and injuries a specific communication will take place between physicians and public health officers or coroners, in which the clinician is supposed to describe the location, the extent and type of injuries to the medical experts of the respective authority (police, court, assurance company). According to Swales'definition it can be called a separate discourse community.[2]

In Hungary, members of that discourse community communicate about diagnostic findings. Injuries have to be described according to statutory regulations on an attestation form. In this document the information concerning the particular injury should be mentioned, according to codified structure. GPs are supposed to use the numbered form, whereas in clinical practice most review findings are processed with computers, in accordance with the codified template, while ensuring compliance with formalities. The question arises whether diagnostic findings should be identified as a genre or register. Since the diagnostic findings are to be understood as a form or a case report with constrictions on structure, format and vocabulary, they can be identified as a genre.

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Members of this discourse community are using a common vocabulary and a specific terminology, which both the clinician as well as the medical officer are aware of. They contain the description of external injuries in Hungarian, the summarizing diagnosis in Greek-Latin technical terminology, and their literal Hungarian translation. This language corresponds with Beaugrandes seven theses on terminology.[1] In the course of medical studies this terminology is not taught. Young specialists learn the rules of this genre and discourse from their experienced colleagues.

When analyzing diagnostic findings of various traumatological wards, one can notice that they do not comply with those rules in every case. Occasionally some important information is missing in the description of the external injuries and/or not mentioned in the Latin summarizing diagnoses. Often times different injuries can be found in the Greek-Latin diagnoses but not in the detailed description in Hungarian, while in some other cases the Hungarian translation contains another type of injury. In such cases, the coroner cannot assess the type and extent of the injuries objectively, and sometimes re-examination of the patient is required. The present case report is

meant to demonstrate how the communication of this special discourse community can be affected by terminology problems. The preliminary study suggests that the cause of difficulties in communication might be the lack of uniform terminology. Some Latin names are translated in various ways. The additional objective of the study is therefore to elaborate a now approach aiming at the unification of technical terminology.

MATERIALS AND METHODS

The following analysis of one diagnostic finding might be a model of a corpuslinguistic research which is in progress. It will describe the methods and goals of a proposed extensive research on this phenomenon. The aim of this research will be to examine further authentic diagnostic findings with corpuslinguistic and contrastive methods and to reveal potential communication problems. 200 authentic findings collected from various Hungarian traumatological wards and institutes of forensic medicine, issued by various physicianswill be analysed. It examines their structure, content and language use. Regarding the origin mechanisms of the injury, the main corpus consists of two groups of findings: Accidents and assaults. In the first phase of research the structure of the diagnostic findings is described as a register of its own authentic examples. In the second phase the corpus analysis follows, which examines the technical terminology of all those findings. Only the external injuries, the findings of instrument-based examinations and the Latin diagnosis plus its Hungarian translation are included in the corpora. The Latin and Hungarian adjectival constructions presenting the descriptions of fractures, bruises and wound types will be analysed with the help of the concordancing program WordSmith 4.0. This makes it possible to comprehend how often a certain word occurs with a certain meaning and which Hungarian equivalent is the most common.

In the third phase the fndings will be analysed manually in a contrastive way. The aim is to observe whether the detailed description of the injuries corresponds to the rules of the register (in relation to an anatomical site, with the corresponding attributes in order to justify the diagnosis). Furthermore, the description of the Latin diagnoses and their Hungarian equivalents will be compared, in respect to the difference between the Hungarian text and the Latin terminology, and whether the Latin and Hungarian diagnoses show the same denotatum. If it can be revealed that the majority of the findings represent these kinds of equivalence problems, and that the coroner must be consulted mainly in those cases, where linguistic verbalisation is misleading, then the solution would be to unify terminology. In that case the goal would be a compilation of a concordance list containing the most frequently occurring terms with their Hungarian equivalents.

RESULTS AND DISCUSSIONS

Hereafter on the basis of an authentic diagnostic finding the steps of the planned research and the factors supporting the hypothesis will be listed.

The structure begins with general circumstances, continues with subjective complaints of patients and ends with objective diagnoses: i.e. it indicates information from general to concrete. Based on Swales'graphics, which demonstrates the information structure of research articles[3], the information structure of Hungarian diagnostic findings could be drawn as in Figure 1.

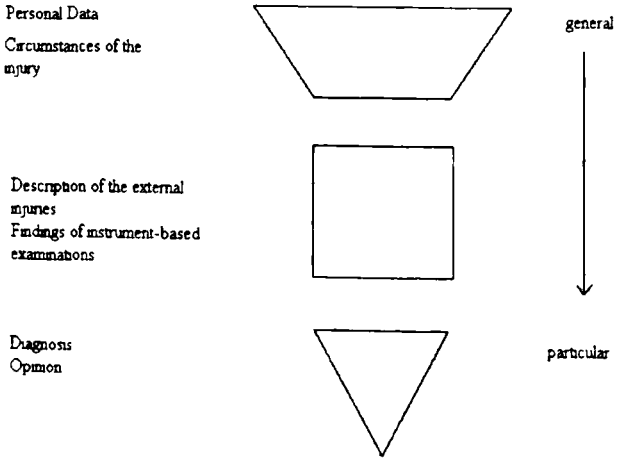

Figure 1. Information structure of Hungarian diagnostic findings

The more one approaches from general to concrete, the more accurate the language use is getting and the more Greek-Latin terms are used.

Example:

State in the examination: On the scalp in the occipital region, a swelling of 3x4 cm size can be seen. On the right palm above the distal end of the second metacarpal bone a superficial slash wound of 1 cm diameter can be seen. At the height of the joint between the first phalanx of the right thumb and the metacarpal bone there is a slash wound of 0.5 cm diameter.
Diagnoses:
Vuln. cont. mani l.d. -Contused wound of the right hand
Vuln. scissum palmae l.d.-Slash wound of the right palm
Cont. nasi bruising of the nose -Contusion of the nose
Cont. capitis -Contusion of the skull

In the detailed description of the injuries there is no special terminology used, even laymen could understand it. There are only few Latin terms to find which describe anatomical relations. The injuries mentionned are two slash wounds whose description correnspond to the rules of the register. Only the wound walls are missing, which would be important for coroners in order to decide whether the wound type is identified correctly.

The summarising diagnosis in Latin lists 4 injuries: a contused and a slash wound, two contusions which are understood in the everyday language mostly as "blue spots". The Latin diagnoses contain a considerable number of abbreviations. Only specialists working in this special field are able to decipher such descriptions. General practitioners in general do not use them. The multiple genitive constructions do not correspond in any case to the morphological rules of the Latin grammar (e.g. „mani" instead of „manus"), but the sense of the whole term is affected by the violation of grammatical rules. The major problem is that injuries listed in the description part differ from those in the summarising Latin diagnoses. The difference between contusion and slash wounds for example is not only a terminological or medical one, but also a legal one. It is a very important question if a wound was caused by mechanical (sharp object) or physical (hand, foot) force.

Another problem is the Hungarian translation of the term "Contusio capitis" (head contusion) which is translated into Hungarian as "contusion of the skull". "Koponya" (skull) is the Hungarian equivalent of the anatomical term" Cranium." This mistranslation could be the result of everyday language use, where the head is often equated to skull. The difference is clear in medicine: bones can not be contused.

In some cases the Latin and Hungarian diagnoses omit the concrete locations of injuries (e.g. contusion of the head in the occipital region, instead of „palm" there is only „hand" or no fixed anatomic point is mentionned), so the final diagnosis will be inaccurate. Otherwhise the injury seems to be thereby less serious (e.g. in other diagnostic findings instead of tibia and fibula fracture just one fracture of the lower leg is mentioned.) The concrete location would be very significant because it could provide information about the origin of the mechanism of injuries. Probably they are not given in Latin because of grammatical difficulties of the terminology. Other diagnostic findings indicate that the Latin and Hungarian names of incised and slash wounds are often inconsistently applied. In the clinical practice it does not seem to be uniform which wound is referred to by which term, even though they are to be found in medical textbooks of Traumatology and Forensic Medicine. If in such cases in the description part the types of injuries are not characterised with the greatest possible accuracy (e.g. the wound walls are not mentioned), the diagnosis cannot be interpreted unambiguously. This type of diagnostic findings is called „not findinglike" by coroners.

CONCLUSIONS

The analysis of this case report may suggest that the non-uniform use of terminology cause problems in the communication between the participants of this specific discourse community. The corpus analysis could prove that these problems occur frequently and need an approach to solve them. In fact, these misunderstandings can also have social consequences in three areas:

1. Financial consequences: the examinations have to be repeated or if insurances receive a false description of the extent of injuries.

2. Legal consequences: a less serious injury will be diagnosed because of the time loss until the second examination.

3. The injured person is exposed to further administrative procedures. The injury victims become victims of a deficient administration.

Relying on the results of the proposed wide-scale research, recommendations could be done concerning the possible ways of unifying the terminology used in diagnostic findings.

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Aphasia from the linguist's point of view

Annamária Győrfi

Aphasia as a phenomenon of speech disorder has been dealt with starting from the second half of the 19th century. Aphasia, also known as aphemia, is a loss of a persosn's ability to produce and/or comprehend language, due to a local injury of the brain responsible for these functions. It is an acquired speech disorder.
There are several methods to analyse it depending on of what we want to learn about it. One of these methods concentrates upon the analysis of speech production, which reflects the location and the particularities of the problem. This can provide the possibility of handling problems of a certain level.
12 aphasic patients at Vac County Hospital, Hungary and Targu Mures University Hospital, Romania accepted to be registered during their speech therapy in 2007. The subjects of this study agreed to reanalyse their speech together in 2009 and provide feedback. This can help them recognize their own mistakes, become more aware of them and by this they improve their own condition.
This study shows that even in cases of severe aphasia speech analysis can help speech therapists in their effort to improve aphasic patients' speaking abilities.
Key words: aphasia, speech analysis, feedback

Aphasia, as described in The Merck Manuals, "is a language dysfunction that may involve impaired comprehension or expression of words or nonverbal equivalents of words. It results from dysfunction of the language centres in the cerebral cortex and basal ganglia or of the white matter pathways that connect them. There is no specific treatment, but speech therapy may promote recovery." [12]

Aphasia was observed and described in many ways through the centuries, but from the linguistic point of view it attracted attention after Paul Broca's (1864) and Carl Wernicke's (1874) findings, which in time led to a better understanding of language processing and classification of aphasia.

Although the first Conference on psycholinguistics was held in Boston, in 1958 and the first linguistic publication appeared five years later [3], we still deal with a relatively new area of linguistics which in spite of the numerous publications raises several unanswered questions.

In case of Broca's aphasia patients can comprehend and conceptualize relatively well, but their ability to form words is impaired. Usually, the impairment affects speech production and writing (agraphia, dysgraphia), greatly frustrating patients' attempts to communicate. Broca's aphasia may include anomia (inability to name objects) and impaired prosody.

In case of Wernicke's aphasia Patients speak normal words fluently, often including meaningless phonemes, but do not know their meaning or relationships. The result is a jumble of words or "word salad". Patients are typically unaware that their speech is incomprehensible to others. A right visual field cut commonly accompanies Wernicke's aphasia because the visual pathway is near the affected area. [12]

These two initial localization models later were classified with others into subtypes of aphasia. Obler and Gjerlow, Lurija, Kolb & Whishaw and many others tried to classify aphasia in subtypes, but as much experts agree or disagree no classification is adequate. Only about 60% of patients will fit in a classification scheme such as fluent (Wernicke's aphasia, Transcortical sensory aphasia, Conduction aphasia, Anomic aphasia) / non-fluent (Broca's aphasia, Transcortical motor aphasia, Global aphasia) /pure aphasias (Alexia, Agraphia, Pure word deafness). There is a huge variation among patients with the same diagnosis, and aphasias can be highly selective. [6,7,10]

An aphasic patient may be able to understand words and sentences while he is not able to produce them or, he may be able to produce certain linguistic forms properly while he is not able to detect the semantics of verbal utterances. [4]

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MATERIAL AND METHOD

Jávorszky Ödön Town Hospital, Vac, Hungary is one of the few institutions where aphasic patients are employed in different rehabilitation programs, as soon as they can communicate somehow with the speech therapist. 11 patients from Vac, Hungary and one patient from Targu Mures, University Psychiatric Clinic agreed to collaborate and have their speech recorded. These patients can be ranked in different subtypes of aphasia. 4 of them suffer from Anomic aphasia, 3 of them from Conduction aphasia, 2 of them from Broca's aphasia, 1 from Wernicke's aphasia (word salad) and 2 of them were not really aphasic (apraxia and dysarthria), but have undergone the same therapy as aphasic patients.

Zsolt R., the only patient from Targu Mures, University Psychiatric Clinic presents the signs of Wernicke's aphasia (word salad), but it can not be ranked clearly in any subtypes of aphasia. This case seems to be unique.

While classifying aphasic patients in subtypes, one of the analyzed features is spontaneous speech. There are several methods of analyzing it depending on what we want to learn about it. One of these methods focuses upon the analysis of speech production, which reflects the location and the particularities of the problem. Concentrating upon their spontaneous speech, a 54'48" recorded material (in 2007) of 5 patients was analyzed in a previous study. [5]

The present paper is a sequel of the above mentioned study as four of the 12 recorded patients were asked to listen to their previously recorded material and give their opinion about it. Recently, they have also agreed to be recorded again for further speech analysis and feedback. This is a new approach, because nothing like this has been done before.

RESULTS AND DISCUSSIONS

When it comes to treating aphasia, the linguist has an important role in it. Comparing Zsolt R.'s case (University Psychiatric Clinic, Targu Mures) who was not included in any rehabilitation program due to the lack of such a Centre in the area with those from Vac, Hungary one can state that his condition is worse than it was before, while those from Vac Hospital mostly improved.

When listening to previous recordings (of 2007) Zsolt R. could remember them, but could not provide a feedback. Unfortunately presently he can communicate less with people around him than he could before. He is more and more locked inside his world.

The other three patients Janos P. suffering from Anomic aphasia, Pal A. - Anomic aphasia and Jozsef H. – Broca's aphasia could provide a better feedback. They listened to their previous recordings and were contented to state that their condition has improved.

Janos P. said that it shows that he was sick, he still had it, but now he could think better, although it is difficult to say things in the same way.

Transcript signs: (1) pause in seconds
(ö) laud pause

Transcript of Jancsi's speech: Jancsi P.: Nem, há:t látszik, hogy beteg voltam, de most is meg van még (.) ez a beszédbe, csak most jobban tudok (ö) (3) gondolkozni, de ugyanúgy nem tudom kimondani.

Pal A. said that listening to it felt really good. He still had the same problems, but maybe not,.....But regarding his speech he felt that it became more fluent and understandable for others.

Transcript of Pal's speech: Hát most, hogy meghallgattam (.) igazán (1) jó érzés volt és (ö) (3) hát még mindig ugye ugyana, vannak problémaim ugyanúgy mint voltak, de azért talán mégsem, ... Míg a (.) a beszélgetésbe én magamon is értem, érzem, úgy hogy (ö) folyamatosabbá válik ez a dolog és hát (ö) (2) mások számára is érthetőbbé válik.

Although Jozsef H.'s speech was more fluent and much better than two years ago he is still not in such condition to convey meaning with his own words as clearly as everyone could understand it.

Transcript of Jozsef's speech: Hát akkor hhm ehh (.) nem is volt, nem is tudom, nem tudom, (.) hát én csak ültem, hogy hogy (.) hogy most mi lesz vagy valami (.) és (.) aztán már utánna már tudom, hogy minden jó.....

Since the last recorded material, the pitch of his voice has improved and this way it has become more expressive. He also used body language during the second recording. When being face-to-face his speech was more comprehensible than afterwards while listening to the recorded material in order to make the transcript. (Chart 1, 2.)

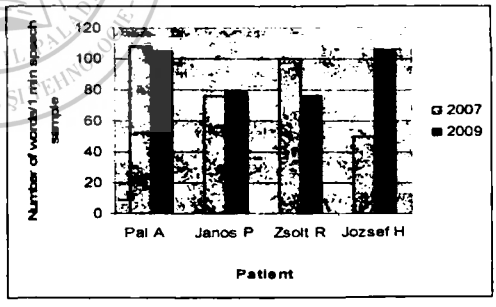


Chart 1. Number of words in speech samples of 1 minute

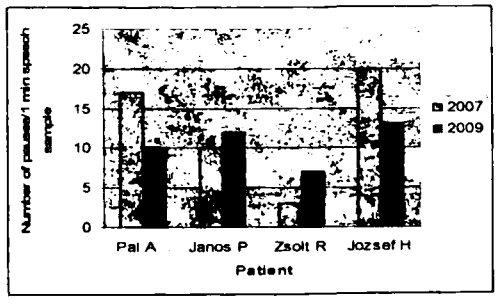


Chart 2. Number of pauses in speech samples of 1 minute

Charts 1 and 2 clearly show that in the case of Anomic Aphasia there was little change. The two patients (Pal A. and Janos P.) were in better condition in 2007 too, but the case of Jozsef H. (Broca's aphasia) shows a significant improvement. Unfortunately Zsolt R.'s condition got worse. He has not been integrated in any rehabilitation program or group dealing with similar problems.

All patients reacted positively when asked to listen to their previous recordings (2007) and willingly provided feedback on it. They were happy to collaborate. This experience shows that they can be helped even more by this method of approach. However the medical and the linguistic approach to some issues may be different this is an area where medical staff and linguists can work and do research hand in hand.

The question is: Is it possible to find out what inside the „black box" is? [5]

Researchers in the medical area are exploring new ways to evaluate and treat aphasia. Different brain imaging techniques help to define brain function, determine the severity of brain damage, and predict the severity of aphasia. [11] The combination of the two relatively new scanning methods, called PET – CT (positron emission tomography and computer tomography imaging) made possible the 4D mapping and visualization of simultaneous structural and identical metabolic information [1]

The following images are illustrative examples of speech activation in the case of brain tumour when aphasia occurs due to deprivation of blood flow in the brain and the modality how other areas of the brain take over the function of speech activation. (Fig.1,2)

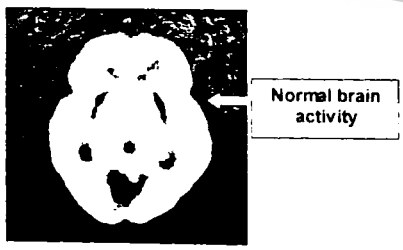
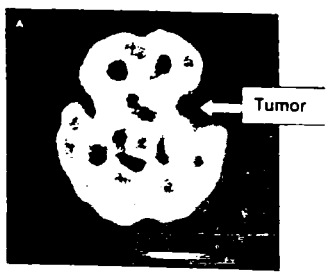
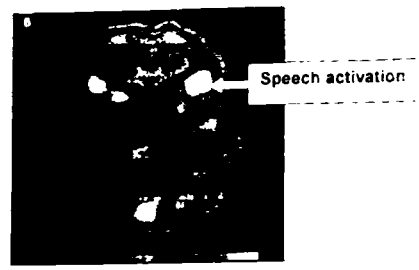


Figure 1 - Normal brain activity





2 Figure: Speech activation in the case of brain tumour

Meta-analyses offer converging evidence to suggest that aphasia treatment brings about meaningful, positive change in language performance relative to untreated controls. [8]

Every individual's brain is unique, and so are the language disabilities one could develop from damage. Every individual with aphasia has a different level of comprehension and production. Aphasia does not affect intelligence in any way. Their brain function does not change beyond their language ability. [5]

Subsequently the author intends to continue this work with the other patients using other methods, too. One of these methods would be the PRAAT speech analyzer, which can measure the intensity of voice, pitch, duration, etc. [2]

CONCLUSIONS

The list of diseases that may cause aphasia is very long, but the most common ones, like brain damage, stroke, brain tumour, head injury, aneurism, multiple sclerosis, etc. in 50% of the cases end up with the patient's death. Those who survive these diseases usually leave the hospital suffering from severe side-effects. Aphasia can be a side-effect that makes patients' life very difficult when returning to their families. They also have the right for living a decent life in the circle of their families and friends. That is why they need professional help.

In conclusion: there is an urgent need to find the way to exploit this phenomenon to provide these patients the "crutches" that best fit them in their recovery.

Special thanks to KatalinNadudvari, speech therapist, who offered her invaluable help to record aphasic patients's speech and Dr. Katalin Borbely for the PET images.

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A linguistic analysis of subjective illness theories in doctor-hypertonic patients interaction

A. Hambuch

Research supports that doctors and their patients miss out on relevant information in their dialogues. This particularly applies for patients' lay illness theories. Data also show that the ratio of hypertonic patients who underwent successful treatment amounts only to 4-27%.[2] This is due to not sufficiently effective medicines, inappropriate choice of medicine, and patients' non-compliance in particular.[3] Although understanding lay theories plays an important role in patients' compliance, they are often regarded as dysfunctional in the doctor-patient communication. While subjective illness theories have been extensively researched from psychological and medical perspectives, there has been relatiyely little research focusing on the linguistic aspect of formulating these theories. In this study data were mainly collected from dialogues between general practitioners and hypertonic patients, in two surgeries in the outskirts of the city of Pécs, Hungary. Patients were randomly chosen, depending on whoever turned up for a consultation that day. Conversations were recorded, transcribed and analyzed using methods of conversation analysis, an established branch of ethnomethodological discourse analysis. Despite the diversity of variables (e.g. length of conversation, difference across sex, doctors' different communication styles, ages and professional background of patients), similarities have been found in the episodic and sequential structure of the dialogues, as well as in the way theories were verbalized in terms of topics and length. Data suggest that medication emerges as a fundamental episode which gives opportunities for patients to verbalise their theories related to cure-control, and as such, determines the conversation. Considering the negative statistics related to patients' medication non-compliance, the importance of medication as an episode requires further research.

Key words: lay illness theories, hypertonic patients, doctor-patient communication, conversation analysis

Successful doctor-patient communication appears to be crucial in maintaining a successful doctor-patient relationship, and successful curing. This research focuses on approaches to the study of successful doctor-patient communication which emphasizes the relationship between professional knowledge and lay theories in doctor-patient interaction. When doctors and patients meet, these two, structurally and logically different types of knowledge interact. This means that successful doctor-patient communication depends on the extent to which doctors' professional knowledge and patients' subjective theories approximate each other.[4] It is also known that in doctor-patient interaction, lay illness theories are often considered disfunctional, and thus they do not emerge as important in these conversations, or if they do, only for a short time.

The reasons for considering patients' lay theories disfunctional in these interactions vary from the lack of time and patient's introverted nature to doctors' deficient communicative competence. This research does not set the aim to explore reasons. It aims to find answers to the following research questions:

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-Which are the components of subjective illness theories that emerge in general practitioners' (GP) and hypertonic patients' interaction?

-When, why and how do these components appear during verbal interaction?

MATERIAL AND METHOD

Data were mainly collected from dialogues between GPs and hypertonic patients, in two surgeries in the outskirts of Pécs, Hungary. Patients were randomly chosen, depending on whoever turned up for a consultation that day. Conversations were recorded, transcribed and analyzed using techniques of conversation analysis. Before starting the conversation, the patients were informed about the aim of the research, and about the way personal data and those concerning their health status were handled.

Patients also gave their written consent to being recorded. Participants' worries about being recorded seemed to be over in the first few minutes, as soon as the discussion shifted to patients' problems.

The results gained from conversation analysis will be later compared to the results provided by a psychological questionnaire, a Hungarian adaptation of The Revised Illness Perception Questionnaire (IPG-R), which the patients filled in after their discussions.

Table I.

Nr.	duration	words	episodes	sections	topics	turns	patient references	doctor references	nurse references
1	01:30	190	2	5	4	19	8	10	1
2	01:34	222	4	6	6	27	*14*	*14*	1
3	01:35	214	4	6	6	30	*18*	*18*	0
4	02:40	404	2	14	10	49	28	30	0
5	02:57	332	4	6	6	34	*17*	*17*	0
6	03:59	604	4	19	15	84	41	40	4
7	05:10	842	5	25	19	84	40	47	3
8	05:59	1075	5	28	19	84	41	47	5
9	06:25	704	8	26	22	96	44	50	7
10	08:15	1153	9	33	26	127	48	71	15
11	09:00	1536	5	39	24	118	72	69	9
12	11:36	1522	5	37	19	137	62	73	14
13	13:33	1418	5	46	24	191	63	99	42

Comparing these results may help to validate, complete, interpret or even refute findings gained through linguistic analysis. Whichever the case may be, this will be informative in itself. Taking into account the stage of research, as well as the limitations imposed by the length of this paper, a full account of findings is not yet possible. In what follows will be presented the preliminary findings that have emerged from the analysis of the first thirteen conversations.

RESULTS AND DISCUSSION

Table1 shows the general characteristics of the conversations, while Figure 1 provides a summary of patients' reasons for coming for a consultation. Columns 8 and 9 of Table I reveal that with two exceptions, the number of verbal responses on both the doctor's and the patient's side was relatively balanced during interactions.

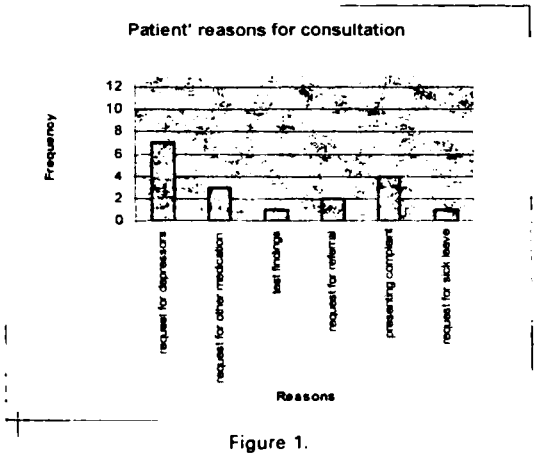

Figure 1.

There were three cases (italicized) when the doctor and the patient participated in the discussion to the same extent. As for the two exceptions mentioned above, one was an 84-year-old hearing-impaired female patient, and the other one was an intellectually disabled young man. As far as reasons for consultation are concerned, the majority of patients showed up for a consultation in order to ask for prescriptions. It is interesting to note that more than twice as many patients came in order to require prescriptions than those who came for a consultation with an actual problem. The linguistic analysis of the conversations focused on two aspects. The first aspect refers to the temporal nature of conversations, which is independent of context. From this perspective, conversations are characterized by the order of turn takings, and by organization into sequences and sections.[3] The other aspect taken into consideration in linguistic analysis refers to the fact that conversations can be divided into larger interaction units.[1] Although these units are determined by the step-by-step sequential order of the surrounding conversation, they still have a complex structure of their own.[3] In what follows these units will be refered to as episodes.

The episodes of the recorded conversations and their frequency of occurance are presented in Figure 2. Figure 3 shows those subjective illness theories which emerge as separate topics within episodes.

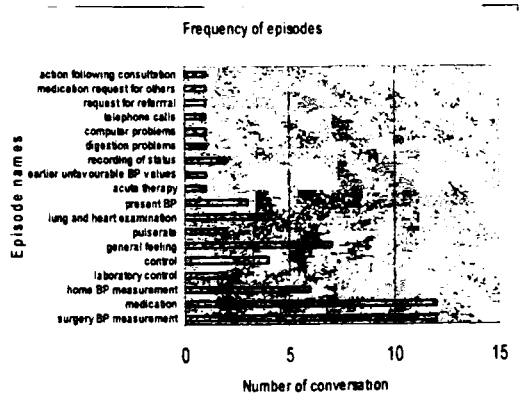

Figure 2.

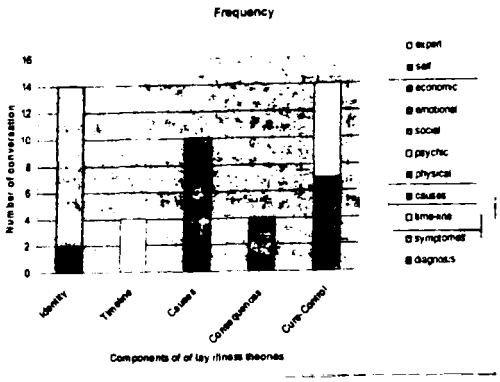

Figure 3.

Figure 3 also provides information as to which components of the lay illness models are represented during verbal interaction. As it becomes obvious from Figure 2, medication and blood pressure measurement were the most common episodes of conversations. Medication was not only the most frequent episode across conversations (with one exception it appeared everywhere), but it was also the most frequently recurring episode within each conversation. Several topics and sequences were found to be typical of medication episodes: out of these, the most typical ones referred to administering previous medication, to introducing new medicines, and to the need for prescriptions. Medication repeatedly appears as an individual episode, but it also emerges as a topic in other episodes (e.g. during blood pressure measurement in the surgery, or lung and heart examination). Thus, medication shapes the conversation either as a separate interaction unit, or as part of other episodes. Related to the importance of medication as an episode or a topic, cure-control and identity were the most powerfully manifested components of subjective illness theories, in verbal interaction. Patients' subjective theories regarding identity (e.g. symptoms experienced when taking certain medicines, side-effects) and cure-control (e.g. the efficiency of medicines) were only represented in connection with certain aspects of medication, for example when discussing the way patients administered medication to themselves, the need for changing medication, or the effects of medicines. It is interesting to note that in the thirteen conversations analyzed, the majority of patients attributed the possibility of cure-control in hypertonia in 50% to themselves and in 50% to doctors. A further finding in either case patients attributed cure-control almost exclusively to appropriate or inappropriate administration of medicines, or, when it came to the doctor's part, to prescribing the appropriate or inappropriate medicine. A healthy diet and physical exercise, as aspects of cure-control, were only mentioned in two conversations.

CONCLUSIONS

Conversations are shaped by medication as a fundamental episode, and by the subjective cure-control theories which are in connection with medication, and which emerge as topics across various sequences. The key importance of medication episodes in shaping discussions is worth considering, mostly if we take into account the negative statistics on patients' medication non-compliance.[2] By extending linguistic analysis to speech acts and turn-taking, further research will be undertaken to describe and explore the relationship between the order of sequences in conversations and the manifestations of subjective lay theories, in case such a relationship exists. This is to be done bearing in mind that the same sequence can bear a different significance at different stages of the conversation. Further research also aims to explore whether the interpretation of lay theories may change in conversations over time, and if they do, what the extent of this change is. Taking things further, the question arises whether it is the structure- or the content-related aspects of the conversation that may influence change, or both. In the long run this research can contribute to increasing doctors' sensitivity and understanding of patients' lay illness theories. Thus, results of the study may also turn out to be valuable in counselling, in particular, and in conveying and assessing information.

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Family interview in organ donation and Hymes speaking model

Melania Gizella Istrate

Organ transplantation has become the only therapeutic method in some end stage diseases, improving the quality of life for thousands of patients. The main cause of losing donors is still the opposition to donation. One issue influencing it is legislation. It is proved that presumed consent countries have higher donation rates than required consent countries, as the donation rate highly depends in the latter ones on the success or failure of the family interview and here communication comes into special attention.

According to Hymes' SPEAKING model, the context wherein words are used is a main criterion in speaking a language correctly. In order to have a successful family interview ending in donation approval, one has to be trained to provide necessary information in the framework of a high quality communication process that has to follow certain steps: steps proposed by Hymes in his SPEAKING model.

Key words: communication, family interview, speaking model

Organ transplantation has become the only therapeutic method in some end stage diseases, improving the quality of life for thousands of patients. Organ transplantation provides present society with huge benefits, as medical needs, unmet so far by other therapeutic procedures, are now solved with excellent results. For example there are approximately 200-300.000 patients with "End stage liver diseases" in Romania, and only 30-50/year benefit from liver transplant. Mortality on the waiting lists exceeds 90%.

A main cause of losing donors is still the opposition to donation. One issue influencing it is legislation (Presumed consent (Opt-Out) / Required consent (Opt-In); Consult Will Registry / Donor Card). Whereas presumed consent gives priority to the recipient, being based on altruism, encouraging positive-solidarity in front of silence of deceased and harming no one, informed/required consent gives priority to the deceased will and represents basis in personal autonomy. It is proved that presumed consent countries have higher donation rates than required consent countries, as the donation rate highly depends in the latter ones on the success or failure of the family interview and here communication comes into highlighted attention.

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MATERIAL AND METHODS

According to Hymes' Speaking model[1], the context in which words are used is a main criterion in speaking a language correctly[2]. The model consists of sixteen components that can be applied to a wide range of discourses such as message form, message content, setting and scene, speaker, addressor, hearer, addressee, outcomes, goals, key, channels, forms of speech, norms of interaction, norms of interpretation and last but not least genres.

The acronym given to this model by the author himself is SPEAKING which comprises the sixteen components within eight divisions.

SETTING and SCENE refer to the physical circumstances and psychological setting of the discourse.

PARTICIPANTS implies addressor, addressee and audience.

ENDS refers to purposes, goals and outcomes of the discourse.

ACT SEQUENCE is given by the form and order of the event that brings up the necessity of a discourse.

KEY: tone and manner

INSTRUMENTALITIES: forms of speech.

NORMS of speech like those of interaction and interpretation.

GENRE refers to the kind of speech act.

According to the research activities undergone in the matter of organ donation by the Spanish team consisting of TPM (transplant procurement management) experts and transplant managers/coordinators (Martí Manyalich -

Universitat de Barcelona, Barcelona, Xavier Guasch - Intensive Care Unit, Hospital General de Castellón, Castellón de la Plana, Ricard Valero - Universitat de Barcelona, Barcelona, Federico Juárez - All For Training. S-L, Alicante, Gloria Páez - Universitat de Barcelona, Barcelona), in order to have a successful family interview ending with donation approval, one has to be trained to provide the needed information within a high quality communication process[2] that has to follow certain steps.

RESULTS AND DISCUSSIONS

After studying the Spanish model[4], I concluded that the model proposed by them for family interview follows the Hymes Speaking model as it follows:

SETTING. The right environment is a comfortable, warmly lit hospital room, with seats for all participants, with tissues placed at hand on the table, a special room where the right to privacy is respected and no interruptions from outside or other barriers are met. SCENE. Breaking bad news is difficult and implies seriousness and deep sorrow.

PARTICIPANTS: transplant coordinator/expert[6] on the one hand and family, relatives on the other hand.

ENDS: to know the deceased will, to evaluate behavioural donor risk factors – in the donor evaluation, to help the family to reduce stress as they experience a critical moment: unexpected death of their beloved one, the overcome the difficulties in understanding Brain Death and the fears about donation.

ACT SEQUENCE. There are three main steps to be respected which have to be carefully prepared by collecting information from doctors and nurses regarding the donor's hospitalization, medical history, useful information about family.

1. Giving bad involves:
   - death communication first
   - emotional, supportive relationship – empathy
   - brain death explanation as a last step

Before telling anything, asking by using open ended questions is crucial. In order to evaluate the organ donor on the one hand and to correct misinformation collected by the family on the other hand it is important to find out about the donor's medical family antecedents, risk factors (sexual habits, drug abuse, smoking, travelling), medical antecedents (previous diseases and treatments) and last but not least what the family knew regarding the donor current treatment and status in the ICU.

Giving bad news is difficult for more reasons: first of all it is the communication of death itself which makes things difficult. Healthcare professionals are not trained regarding how to lead a highly qualified communication process. It must be lead by an expert, a TPM according to the Spanish model[5]. (Transplant Procurement management).

Last but not least, there is a fear of getting blamed, unleashing a reaction, feeling incompetent or feeling embarrassed.

2. Time for grief - family meeting make sure that relatives or partners understand the irreversibility of the process – brain death is death, time for grief not too long – depends on stability of the possible donor.

3. Donation request as an opportunity for the family, cultural awareness about death, ownership of body, explain process of organ removing.

KEY. It is known that more than 60% of the whole communication process is provided by nonverbal communication. Silences, eye contact, physical position (no crossed legs or arms), physical contact (a reassuring pat showing that the expert cares), use of gestures, warm and low tone of voice are key points in addressing their emotions, in observing and identifying the experienced emotion and therefore in establishing an empathic relationship with the family approached.

INSTRUMENTALITIES. Be clear and concise, use the listeners' own vocabulary, verify degree of comprehension, follow their rhythm of comprehension.

NORMS. A special room where the right to privacy is respected and no interruptions from outside or other barriers are met. Open ended questions, active listening, summarizing, metaphors, reasoned language are to be used in order to collect any useful information about the organ donor medical history and avoid so any biological risk factors, to overcome the difficulties met by the family in understanding Brain Death and their fears for donation.

GENRE. The family interview is not a conversation. The communication process has to persuasive and convincing, bringing

1. Arguments for requesting Donation such as:

-Solidarity: social – we are all involved, group – people on waiting list, individual – someone known, family or friend, on dialysis

-Utility: Death useful to someone, a form of continuity of life for other people

-Reciprocity, Generosity: positive arguments – elevate the image of the deceased, we would do the same, tomorrow it could be for us

-Facilitation: help with formalities – ICU visit, legal aspects, funeral home; provide privacy and intimacy

-Strategies to face family refusal

2. Deceased did not wish to donate - trying to find if it is true

-Family not knowing deceased will to donate - arguments of solidarity, utility, reciprocity and generosity

-Not understanding brain death - explaining brain death

-Fear of the integrity and image of the corpse - explaining the process, assuring respect and care of the corpse

-Religious objections - offering the opportunity to consult a religious leader

-Dissatisfaction with the health assistance - accepting complains, separating the unfavorable donation context: receptors are not guilty

CONCLUSIONS

The present study was intended to analyse the Spanish model of family interview in organ donation[7] through Hymes Speaking model. The findings suggest that the Spanish model follows the speaking model proposed by Hymes, which makes it more successful in required consent countries as the European Training on

Organ Donation DG SANCO project (with 16 European countries) emphasized.

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Molecular-level knowledge representation in oncological descriptions

A. Szelényi

Linguistic representation of newly discovered entities has always posed a challenge on scientists. Professional description of the phenomena observed varies on a wide scale. Describing the morphological features seems to be the most obvious way that has been available ever since scientific descriptions were first produced. The rapid development in the quantity and quality of scientific research and the ever-increasing depth of observations necessitated the introduction of new aspects, including functional description. The emphasis from the primarily static descriptions of macroscopic and microscopic investigations is gradually shifted to primarily dynamic molecular-level descriptions [5]. While scientific descriptions at the former levels have been prepared for several centuries, the introduction of the molecular level of observations resulted in the emergence of function descriptions. The problems related to the discovery of an increasing number of factors, as well as the increasing difficulty of physical observations due to the small sizes the emphasis is more and more frequently laid on the systemic representation of the role, effect and function of individual constituting elements. This trend is also observable in the currently developing nomenclature of molecular-level oncological research. A constantly growing mass of scientific data to be described will be presented as a complex system, with regard to the fact that in support of a fast international data exchange the prevalence of English-language nomenclature tends to become stronger and stronger even in national-level biomedical research.

Key words: *description, molecular-level, nomenclature, function*

It has always been a great challenge to describe new scientific knowledge by appropriate linguistic means. A proper selection and classification of the observed features under study are required to find the best terms for an unambiguous description. Application of characteristics generally accepted by the experts ensures that the scientific circle such as those of oncology or other population of interest could be best informed on the topic in question. In particular, macroscopic and conventional microscopic observations are based on morphological, topological and structural characteristics rather than on other means. The most appropriate name for the formulation of a phenomenon does not necessarily serve a precise characterisation; instead, just one or two features are represented by that name such as the one related to its form of object to be described. For example, the term "cancer" coined by Hippocrates might have originated from the similarity between the form of an advanced neoplasma and that a cancer (shore crab) characterized by a central swelling of its body and the claws stemming from it. The macroscopic or conventional microscopic levels are basically static descriptions of the phenomenon in question be it either the tumour itself or the forms recognisable on its tissue section, respectively. A microscopic description of a tumour mass may include some dynamic changes occurring with time, but that aspect generally cannot be recognized on the bases of its name. The development of a tumour is very difficult to judge by looking at it, rather some static external characteristic of it may appear in the morphological description at a certain stage of tumour growth. Also, be it either a histological or cytological examination, the result looks like a snapshot[1] along the timescale of its dynamic state. These types of studies concentrate on morphological-structural features allowing precise description and differentiation of different types of forms. The analysis consists of demonstrating limited number of factors sometimes defying their easy discrimination by visual means. Such materials of study may be homogeneous

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with limited possibility for differentiation of its parts, but as a result of technical improvements more and more details have been possible to decipher. With the availability of more elaborate methodology and equipment, clear-cut simple description of the morphological pictures has become more difficult to do, a situation resulting in a limitation of the number of experts able to analyse these data successfully. As a result of these developments, a rapid specialization has been mandatory: in fact, the exclusive or even dominant visual nature of these investigation has largely been replaced by molecular approach leading from the conventional static thinking to functional reasoning.[4] Also, the more isolated description of the different forms and phenomena has been replaced by the analysis of the multitude of observed, inferred or hypothesized relationships among the factors under study[6]; the result has been the visualization[5] of links or even nets of the contributing players having either reinforcing or contradicting complex relationships with each other.

MATERIALS AND METHODS

It was aimed at to investigate oncological descriptions of molecular nature. The texts to be studied here belong to most recently published relevant descriptions of oncological phenomena appearing in Hungarian textbooks. Molecular studies aimed at not only to experts in the field but also to medical students and interested laymen can be regarded as up-to-date. It should be emphasized that the texts under study belong to molecular oncology, an interdisciplinary medical speciality being understandable only to those familiar in oncology, genetics and molecular biology. The vocabulary and the abbreviations used by this discipline have largely originated from English[1,2] and therefore the texts are not easy to understand by Hungarian readers. An easier orientation in this field may be assisted by the systematic use of short and full English expressions[2] in most of the passages studied. Even some letter-figure combinations are applied to identify certain investigation or phenomena in the texts.

RESULTS AND DISCUSSIONS

Texts describing phenomena of molecular oncology nature deal with characteristics of cell proliferation form several aspects, in that conventional morphological, some closely related approach and hierarchically formulated descriptions can all be all found in the texts studied together with some attempts to characterize the given phenomenon in its relation to the environment of the tumour in question (e.g. to the extra cellular space). The relationships among the different elements are analysed according to their site of (receptor), actions (activating or regulating effects), to their stimulating (enhancing) or inhibiting (repressing) nature as well as to the emphasis on other aspects (coupling or association) or factors (integrins, transmitters). The different "players" of this highly complex system may be looked upon as single effects versus complex biochemical mechanisms[8] (e.g. protease activated receptor, PAR). The subtopics just mentioned may appear on the level of vocabulary (names), in concentrated or hidden form or just as abbreviations. The Hungarian texts often use English expressions[1] (turnover, loop, high grade glioma) or wording (expresszálódik, amplifikált, juxtamembrán, permeabilitás), even when appropriate Hungarian words are available. In several descriptions it seems as if tumours or cancers would be complex living beings (creatures) capable of independent thinking or even of action and making decisions for their own survival. As opposed to this, factors acting against cancer development are looked upon as "troops of enemy" fighting against cancer development and possessing conflict of interest in cancer business. As a result of this, an outline of a complete war appears in the background characterized by individual fightings between opposing factors and mechanisms. This personalization appears to play an important role in the understanding the pathological process behind cancer development and its visualization.[5] In fact, the latter aspect helps bring non-medical people closer to the basic process of tumour development and help them appreciate this otherwise extremely complex phenomenon. The process of observable and even measurable cell proliferation shows a spatial and temporal dynamics that originates from functional molecular characteristics of complex interactions among the large number of factors. Thus, a system of functional net of interactions emerges.[7,9]

CONCLUSIONS

As a new field, molecular oncology has yielded a novel way of visualizing this group of diseases. The ever deeper level of studies has resulted in an exponentially increasing number of elements[1], the description of which necessitates the use of a new vocabulary and abbreviations found in the relevant literature published in Hungarian. This constraint has largely been induced by the organization of new international studies of molecular oncology, by the rapid appearance and availability of information in this field and by the ever increasing need for the national (Hungarian) application of successful diagnostic and therapeutic possibilities based on large international databases. There is a trend for the dominance of functional approach and the need for knowledge of mechanism[4] of action in oncology that has resulted in an increased usage of novel vocabulary in publications.[1] As a result of lack in understanding the basic mechanisms of cancer development the metaphoric usage of some terms still prevails resulting among others in personalization of the natural players in tumour genesis and defence. The dynamic nature of tumour development[5] and the success in deciphering more and more contributing factors have resulted in a shift from a purely physical (morphological) approach of study used to be dominant in the past into a more functional molecular one.

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CD 105/ smooth muscle actin double immunostaining expression in liver metastasis from colon carcinoma

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The liver is the most common and critical site for the development of colon cancer metastases. Tumor angiogenesis in liver metastasis from colon carcinoma is a controversial subject. Liver microenvironment, immunophenothypical and morphological particularites of hepatic vessels are only few aspects wich establish difficulties in cuantification of tumor vascularisation from liver metastasis. The aim of this work was to demonstrate the value of double immunostaining for endothelial and perivascular cell to discriminate mature from immature blood vessels and to describe their distribution in peritumoral and intratumoral areas. Our study included 12 liver biopsy from patients who present metastasis from previous diagnosis colon carcinoma. We used haematoxylin-eosin method, for the pathological diagnosis. We applied a double stain method using CD105 and smooth muscle actin antibodies. Concerning CD105/smooth muscle actin expression we notice a highest number of vessels CD105 positive/SMA- in peritumoral areas compare with intratumoral areas in all cases of liver metastasis. In liver metastasis from well differentiated colon carcinoma we found CD105+/actin positive vessels in peritumoral areas suggesting preexistent activated vessels. In all cases CD105 expression had an intense expression in sinusoidal endothelial cells and absent in vessels from portal spaces.

Key words: *colon carcinoma, liver metastasis, CD105, smooth muscle actin*

The colon cancer is one of the third most frequent cancer in both men end women over the age of 65. Over the time, 40–50% of newly diagnosed CRC patients will develop metastatic disease.[12] The liver is the most common and critical site for the development of colon cancer metastases. The 5-year survival rate for patients with metastatic CRC is 5–30%.[18] It is well established that angiogenesis is necessary for the growth and metastasis of tumors. Endoglin (CD105) is a receptor for the TGF-b1 molecule that is upregulated in tumor neoangiogenesis.[1] The endoglin antibody binds preferentially to the activated endothelial cells that are involved in tumor angiogenesis, but expression is weak/or negative in vascular endothelium from normal tissues.[2,10] CD105 is weakly expressed on non-endothelial cells of different histotype, like activated monocytes, macrophages, fibroblasts, melanocytes and vascular smooth muscle cells.[4,5] CD105 expression is strong and restricted to capillary endothelial cells in cancer lesions (>73%). In contrast, expression of CD105 was seen in less than 20% of non-cancerous areas in the same organs. CD105 is expressed specifically in the tumor angiogenesis of brain, lung, breast, stomach, and colon cancers.[11]

Immature vessels, which are not covered by pericytes, are irregular and leaky. Smooth muscle cell actin (SMA) can be used to highlight perivascular cells. Immature neovascularization was observed in poorly differentiated tumors and was correlated with metastasis, resulting in a poorer prognosis. Taken together, not only microvessel density but also vascular maturation seemed to be crucial factors for colon cancer patients.[20]

The formation of liver metastasis from colon carcinomas is initiated by the extravasation of a circulating tumor cell within the liver. After tumor enlargement progresses to a point beyond which angiogenesis is necessary to sustain growth, neovascularization occurs in a stepwise fashion[16] and proliferation of sinusoidal endothelial cells serves as the primary basis for angiogenesis.[8] In the peritumoral region, sinusoidal capillarization has been found to occur through a process similar to that seen in

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hepatocarcinogenesis.[15] The aim of this work was to demonstrate the value of double immunostaining for endothelial and perivascular cell to discriminate mature from immature blood vessels and to describe their distribution in peritumoral and intratumoral areas.

MATERIAL AND METHODS

Our study included 12 liver biopsy from patients who present metastasis from previous diagnosed colon carcinoma. Resected liver specimens obtained by open surgery were fixed in bufferred formalin for 48 hours and parrafin embeeded. Five thick step sections were performed from each block. Initial sections were stained with routine haematoxylin-eosin method for histopathologic conventional diagnosis. We applied a double stain method using CD105 and smooth muscle actin antibodies. On dewaxed and rehydrated slides we performed endogenous peroxidase blocking with 3% hydrogen peroxide for 5 minutes followed by pretreatment with Proteinase K for 15 minutes at room temperature. Incubation with primary CD105 antibody, clone SN6h from DakoCytomation for 1 hour (dilution 1:10) preceeded the first application of avidin-biotin working system LSAB+-HRP and visualisation with 3,3'diaminmobenzidine as chromogen. After 30 minutes incubation with smooth muscle actin ready to use antibody (clone 1A4, Dako), the LSAB+-HRP working system was used, followed by visualization with AEC chromogen for 10 minutes.

RESULTS

On CD105/ smooth muscle actin stained slides we noticed the highest number of vessels CD105 positive / SMA- in peritumoral areas in both cases of liver metastasis from poorly differentiated colon carcinoma. In the intratumoral zone we observed that the number of CD105 positive vessels was low compared with the peritumoral area. Concerning differentiation and maturation stage there are imature, without lumen, unperfused and intermediate vessels – perfused, with narrow lumens. In liver metastasis from moderately differentiated colon carcinoma we found almost the same aspects: a high number of CD105 positive vessels in the peritumoral areas but smaller number than similar zone from poorly differentiated colon carcinoma; intratumoral we noticed a reduce number of vessels compared with peritumoral area In five from eight cases (62,5%) of liver metastasis from well differentiated colon carcinoma we found CD105+ / actin positive vessels in peritumoral areas suggesting preexistent tumoral activated vessels. In all cases CD105 expression was intensely in endothelial cells from hepatic sinusoids close to the tumor and absent in vessels from portal spaces.

DISSCUSION

In human liver, CD105 (also known as endoglin) is present on sinusoidal EC next to neighboring of portal veins, whereas CD34 is expressed predominantly in the periportal region.[9,17] The distribution and expression of HIF-1alpha in liver tissues with hepatocelular carcinoma revealed that positive staining was located in the cytoplasm and/or the nuclei of tumor cells and hepatocytes. In general, the intensity of HIF-1alpha staining in the non-tumor tissues was higher than in tumor tissues. In the portal area of cirrhosis, the expressions of HIF-1alpha in the bile duct and the vessels were negative. The levels of CD105 protein, mRNA and promoter activity can be up-regulated by hypoxia via the HIF-1 complex, which binds a functional consensus HRE in the endoglin promoter.[13] In general, the intensity of $VEGF_{165}$ staining in the non-tumor tissues was higher than in tumor tissues. $VEGF_{165}$ signals were also present in endothelial cells. CD105 mRNA correlated with $VEGF_{165}$ in non tumor and tumor tissue.[3] The endothelial cells lining the liver sinusoids demonstrates microheterogeneity.[19] In rats, caveolin-1 expression is increased in periportal sinusoidal ECs. In liver metastases vessels are lined with sinusoidal endothelial cells. Identification of a specific cell type involved in the formation of the stromal compartment of tumors has important implications. Sinusoidal endothelial cells express well-characterized surface receptors and differ morphologically and metabolically from large-vessel endothelia.[7] In our study all cases of liver metastasis from well, moderately and poorly differentiated colon carcinoma present CD105 positive in sinusoidal endothelial cells compared with portal area where CD105 was negative. Saad et al[10] showed that assessing microvessel density using endoglin in the colorectal cancer might be a valuable parameter for predicting patients having an increased risk of developing of metastatic disease. Endoglin, by staining only the proliferating microvessels in colo-rectal carcinoma, is a more specific and sensitive marker for tumor angiogenesis than the other commonly used panendothelial markers. Endoglin staining also showed prognostic significance with positive correlation with the presence of angio-lymphatic invasion, lymph node metastases, tumor stage and hepatic metastases. A study of Stessels et al[14] shows that liver metastases of breast cancer mainly co-opt sinusoidal blood vessel during their growth, in contrast to most of the colorectal cancer metastases, that expand with concomitant hypoxia-driven angiogenesis. The vessel-co-opting replacement growth pattern and the angiogenic desmoplastic growth pattern have also been observed in animal tumor models. Fukumara et al[6] using intravital fluorescence microscopy were able to perform direct observation of solid tumors grown in the liver and demonstrated that vascular density in the center of neoplasia was lower than in the periphery. Tumor cells can penetrate immature microvessels more easily than mature microvessels. In the cases of liver metastasis from poorly differentiated adenocarcinoma of colon we noticed that the majority of the vessels expressed CD105+/actin- in both peritumoral and intratumoral areas. This finding is in according with study of Yonenaga et al.[20] which demonstrate that immature

neovascularization was observed in poorly differentiated tumors and was correlated with metastasis, resulting in a poorer prognosis.

CONCLUSIONS

Double immunostaining CD105/ actin is useful for differentiation between mature and immature vessels in liver metastasis from colon carcinoma.

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Neuronal markers in the human vomeronasal epithelium

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Introduction: The receptor structure of the vomeronasal system (VNS), the vomeronasal organ (VNO), is located bilaterally above the base of the nasal septum. There are ongoing controversies regarding the functionality of this system in humans. Our aim is to document the existence or absence of receptor like components in the organ's epithelium using immunohistochemistry.

Materials and methods: We studied 4 fetuses of different gestational ages, from the Department of Anatomy, University of Medicine and Pharmacy, Tg-Mures. Following the identification of the VNO, tissue samples were dissected, sectioned and paraffin embedded. The markers used: cytokeratin 7 (CK7), neurofilament (NF), CD 57, N-cadherin, calretinin, connexin 43.

Results: The presence and microscopic structure of the fetal VNO was confirmed. All markers resulted in positive reactions.

Discussion and conclusions: CK7 positivity confirmed the VNO's epithelial structure. Connexin 43 may indicate gap-junction type intercellular connections. The organ contains neuronal components confirmed by calretinin, N-cadherin, neurofilament and CD 57 positivity. This may indicate a functional receptor epithelium in the human fetus.

Key words: vomeronasal organ, fetal, human, immunohistochemistry

The vomeronasal organ (VNO) is the peripheral sensory organ of the accessory olfactory system. In most mammals the paired organs are located at the base of the nasal septum. There are numerous examples of vomeronasal involvement in chemical communication, although pheromone communication is not the exclusively a vomeronasal function. The existence of a VNO in the human embryo similar to the VNOs of other species is well documented.[3] It contains bipolar cells similar to the developing vomeronasal sensory neurons of other species and also generates luteinizing hormone releasing hormone (LHRH)-producing cells as in other species.[4]

There are ongoing controversies regarding the functionality of the human vomeronasal system. The human VNO not only differs significantly from the VNO of other species, but also there are contradictory reports regarding its postnatal persistence or involution.

The present study aims to bring further proof attesting the presence and persistence of neuronal elements in the VNO epithelium of the human fetus.

MATERIALS AND METHODS

We processed the nasal septa of 4 human fetuses of different gestational ages. The measurement of crown-rump length has been performed for later reference (mean CRL was 14.5 cm). The fetuses were all spontaneous abortions and were processed at the Pathology Department of the Clinical County Hospital Targu-Mures.

The nasal septa were removed by macroscopic dissection and the obtained tissue fragments were oriented in the frontal plane and sectioned macroscopically. The resulting 4-5 mm thick blocks were paraffin included and 5 micrometer histological sections were obtained.

Following positive identification of the VNO we proceeded to immunohistochemistry, partially performed at the Pathology Department of the Clinical County Hospital Targu-Mures, and in part at the 1st Pathology and Experimental Cancer Research Institute in Budapest.

We used the following markers: cytokeratin 7 (CK7), neurofilament (NF), CD 57, N-cadherin, calretinin and connexin 43. The section were dewaxed and rehydrated, followed by endogenous peroxidase blocking using 1.5 ml H_2O_2 and 90 ml methanol. For the CK7 reaction we used the streptavidin-biotin method, with steam cooker and citrate antigen retrieval.

For the rest of the markers we used the Novolink kit and protocol (Novocastra Laboratories, Ltd.), with steam cooker and Tris-EDTA antigen retrieval. The chromogen was DAB in all immunohistochemical reactions.

The antibodies and dilutions were as follows: connexin 43 (Cell Signaling) 1:200; N-cadherin (IAR 06, Novocastra) 1:600; calretinin (DAK- Calret 1, Dako) 1:4; neurofilament (2F11, Dako) 1: 400; CD 57 (NK-1, NovoCastra) 1:30; cytokeratin 7 (OV-TL 12/30, Lab Vision) 1:50.

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RESULTS AND DISCUSSIONS

The VNO is found throughout fetal development, although its presence is not constant. In our search for suitable specimens, we found an approximately 40% incidence of the organ among the examined fetuses.

The histological structure of the VNO reveals a blind epithelial tube located on the side of the nasal septum, with an epithelium that resembles respiratory epithelium. It has an average thickness of approximately 50 microns, and displays several layers of nuclei. These are grouped more towards the basal membrane, leaving a luminal band of nuclei-free cytoplasm. There are isolated groups of microvilli scattered on the apical surface of the epithelium.

Connexin 43 showed a specific positive reaction, located throughout the cell membranes of the VNO. The respiratory epithelium had similar positvity (Figure 1).

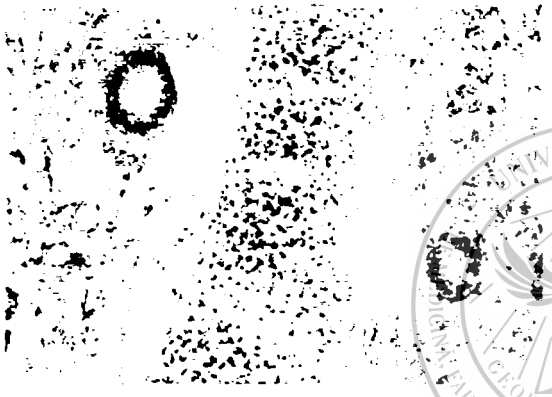

Figure 1. Connexin-43 reaction

N-cadherin also had a positive reaction, located mainly in the basal cells of the epithelium, and also in the luminal nuclei-free zone. The reaction seemed less intense in the middle part of the epithelium, where the most numerous nuclear layers were located. Several N-cadherin positive bands cross the epithelium from the surface towards the basal membrane (Figure 2).

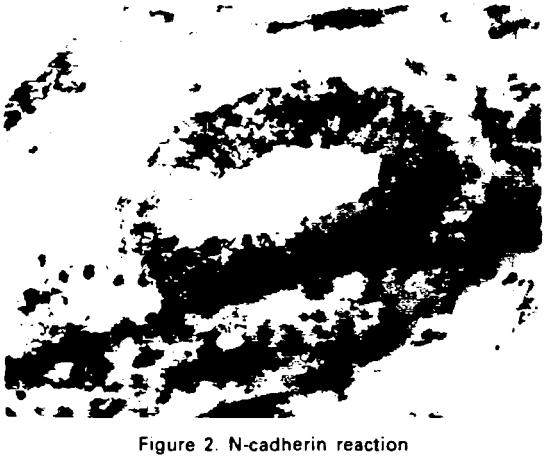

Figure 2. N-cadherin reaction

The positive immunohistochemical reaction for calretinin had a localization very precisely confined to the basal layer. These cells showed a very intense positive reaction, with a few more positive cells scattered through the upper parts of the epithelium. In contrast, the middle and upper layers had no noticeable positivity (Figure 3).

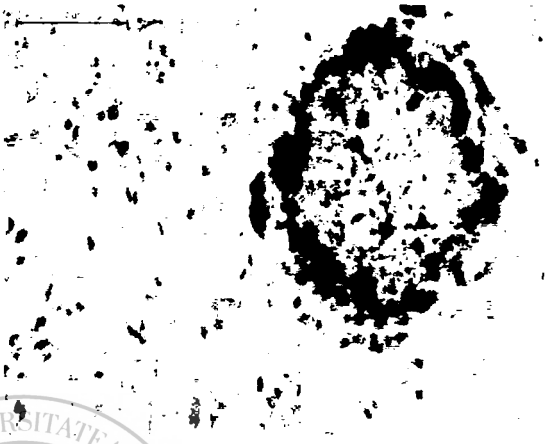

Figure 3. Positive reaction for calretinin

The neurofilament positivity was confined to a small number of cells scattered through the whole epithelium. Overall these positive cells represent a reduced density, but still, there are positive cells that span from the surface to the basal membrane, but there are no axons leaving the epithelium (Figure 4).

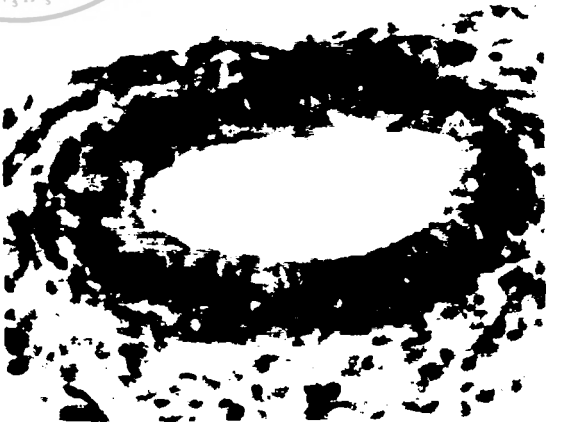

Figure 4. Positive reaction for neurofilament

CD57 positive cells were found in the basal layer of the VNO epithelium, as well as around the luminal surface in a lesser extent (Figure 5).

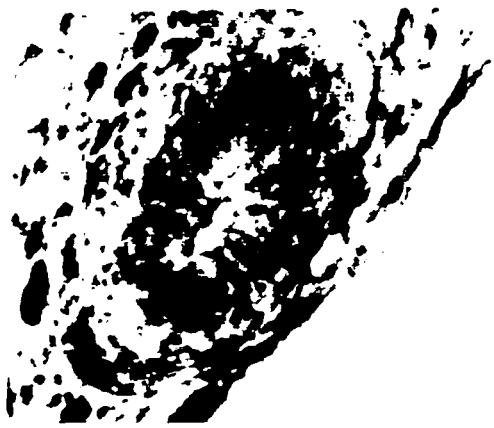

Figure 5. CD57 reaction

The CK7 reaction revealed intense membrane positivity extending to the entire epithelium, with a continuous positive reaction at the apical surface (Figure 6).

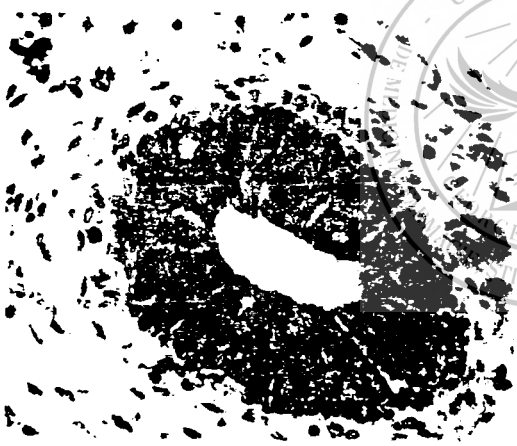

Figure 6. Cytokeratin 7 reaction

Connexin 43 (Cx43) is a member of the large family of gap junction proteins. Gap junction communication is important in development and regulation of cell growth.[6] Our results suggest the presence of gap junction type intercellular adhesions in the epithelium of the VNO.

The most widely distributed neuronal cadherin is N-cadherin, whose distribution changes during development. In cultured hippocampal neurons, N-cadherin is progressively lost from inhibitory synapses during development and preferentially retained at excitatory synapses.[2]

Calretinin and calbindin-28 kDa belong to a family of low molecular weight CBPs expressed in the retina and nervous system of vertebrates.[5]

Neurofilaments belong to the family of intermediate filaments and are structural elements of the neuronal cytoskeleton in an interconnection with actin microfilaments, microtubules and other intermediate filaments. This antibody labels neurons (axons) of the central and peripheral nervous system.[7]

By obtaining positive immunohistochemistry reactions for N-cadherin, calretinin and neurofilament, we tried to demonstrate the presence of neuronal elements in the fetal VNO epithelium. Studies of the adult human vomeronasal epithelium have reported the presence of bipolar cells that are like the vomeronasal neurons of other species. These cells contain neuron-specific enolase (NSE), a marker substance characteristic of neural cells.[9,10] There are no reports suggesting olfactory marker protein (OMP) positivity of the human VNO. This is a characteristic marker of chemosensory cells.

Previous studies described axons in the epithelium, but not in continuity or in synaptic contact with epithelial cells.[8] Our neurofilament results confirm the absence of axonal contacts.

Besides its well known role as a lymphocyte marker, CD57 is also expressed by nerve fibers, for example in epithelial sheath neuroma. We demonstrated CD57 positive cells in the basal VNO, possibly indicating axonal projections, although this positive reaction could also mean the presence of neuroendocrine cells.

Cytokeratin positivity has been demonstrated previously in the VNO epithelium[10], which is confirmed by our results.

CONCLUSIONS

We concluded that the epithelial structure of the VNO has been confirmed by the cytokeratin reactions, and intercellular adhesions are probably mediated by gap junction type connections. The presence of neural elements in the developing fetal epithelium is confirmed by the positive neuronal markers. The lack of neurofilament positive axonal projections raise a question regarding the superior connections of the VNO, and it pushes the interpretation of CD57 positivity towards neuroendocrine origin.

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An assessment of romanian internet users' perception of the preventive and curative properties of food and dieting

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The increased interest of public opinion toward nutrition as a means for prevention and treatment of diseases is a megatrend of the contemporary society. Our synchronous Web-based survey, was conducted on a sample of 450 Internet users from Romania. The 9 item online questionnaire aimed to assess how healthy and sick people perceive the preventive and curative properties of food in general and in particular the vegetarian diet. The mai results show that 39.6% of the respondents believe that diet can prevent and cure any diseases, including cancer. Also, in case of disease, 13.8% of the respondents said the pacient should refuse conventional treatments in favor of a dietetic cure.
***Key words**: diet therapy, vegetarian diet, conventional treatment, alternative treatment*

The unprecedented increase in the public's interest towards nutrition as a means to prevent and treat diseases is a megatrend of the contemporary society since the middle of the XXth century[12]. The impact of food and nutrition on health and disease represents a major concern for bout medical professionals and general public [6,16]. Nutrition researches are discovering more and more evidences about the protecting effect of healthy nutrition and nutrients.[18,19] Diet-based therapies are one of the most popular complementary and alternative medicine (CAM) therapies. Contrasting with the enthusiastic view reflected by the media, the scientific proofs supporting the benefits of food regimens as a therapeutic mean are still modest.[17]

Our study attempted to evaluate the perception of Romanian Internet users regarding the preventive and curative properties of food in general and in particular the vegetarian diet, and the connections between the opinions of the subjects and their attitude towards conventional medical treatments.

MATERIAL AND METHOD

Our study was designed as a descriptive cross-sectional study. The data were collected using a synchronous Web-based survey with 9 questions. The questionnaire was available online at www.sanatateplus.net from June 16 to August 22, 2008 (over 2 months), and was advertised on several medical and general interest websites and forums. Participation was voluntary.

We obtained 451 sets of answers, out of which 450 were included in the study as being eligible. The data were statistically analysed using GraphPad software.

The structure of the group, according to age, gender, marital status, ethnicity, religion, school education and residency is detailed below (Table 1).

All counties of Romania were represented. Bucharest and Mures county registered a high, relatively disproportionate number of subjects (2.4% and 15.6%) compared to other counties. There were 30 persons who live abroad.

RESULTS

1. The answers to the first question (Figure 1) showed that 39.6% of the respondents attributed total preventive and curative properties to food; 34.9% considered that food regimens can prevent and cure only mild diseases; 23.6% believed that nutrition has only preventive properties against some diseases, and 2.0% thought that food plays just an insignificant role in prevention and treatment of ailments.

2. The analysis of the answers to the second question revealed that out of those respondents who believe that foods have curative properties, 23.4% considered that the most efficient method was the raw fruit and vegetable juice cure, 21.3% the total vegetarian diet and, 20.8% the ovo-lacto-vegetarian diet. (Figure 2) The total vegetarian diet, under different

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Table I. Distribution of subjects according to socio-demographic criteria

Social criterium	Nr. subjects	Frequency (%)
Gender		
Male	293	65,1
Female	157	34,8
Marital status		
Single	152	33,8
Married	250	55,6
Divorced	34	7,6
Widow	3	0,7
Consensual union	11	2,4
Ethnicity		
Romanian	408	90,7
Hungarian	34	7,6
German	3	0,7
Other ethnicity	5	1,1
Religion		
Orthodox	193	42,9
Roman – Catholic	15	3,3
Greek – Catholic	5	1,1
Protestant	67	14,9
Neoprotestant	130	28,9
Other religion	33	7,3
No religion	7	1,6
Residency		
Very large city (over 200.000 inhabitants)	174	38,7
Large city (100.000-200.000 inhabitants)	129	28,7
Small city (30.000-100.000 inhabitants)	78	17,3
Very small city (under 30.000 inhabitants)	26	5,8
Commune	32	7,1
Village	11	2,4
Total	450	100

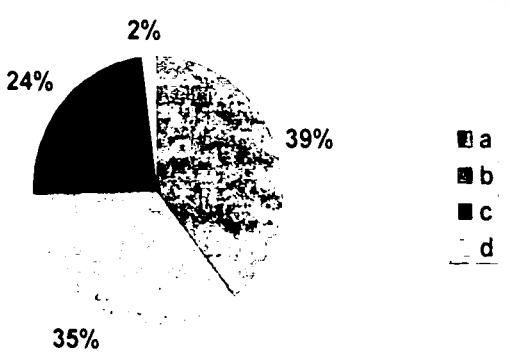

Figure 1. Answers to question no. 1

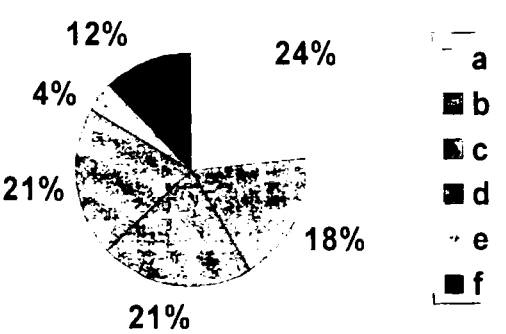

Figure 2. Answers to questions no. 2

Legend for figure no.1

Do you consider that food:
a. can prevent and cure all diseases (including those considered incurable by medical professionals)
b. can prevent and cure milder diseases
c. can have only a preventive effect in some diseases' case
d. has a negligible role preventing and curing diseases

Legend for figure no. 2

In case you considered that diseases can be cured through diet, which diet do you think is more efficient?
a. raw fruits and vegetables juice diet
b. strict vegetarian diet (only raw vegetables, not cooked)
c. strict vegetarian diet(including vegetables, no meat, milk or eggs)
d. egg-milk vegetarian diet (vegetables, milk, eggs)
e. meat diet, with vegetables and not cooked animal products
f. any vegetarian diet

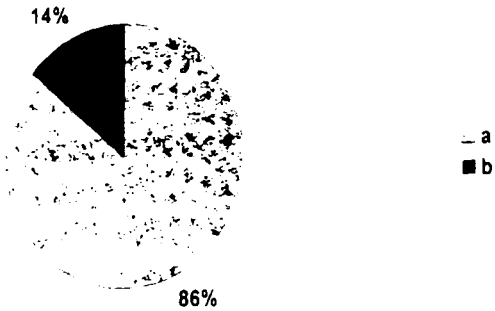

Figure 3. Answers to question no. 3

Legend for figure no. 3

If a person is on a diet to cure a disease, he/she should:
a. undergo,in parallel, a medical treatment recommended by a specialist or the general practisioner
b. not accept any other treatmet – medical, surgical or X ray

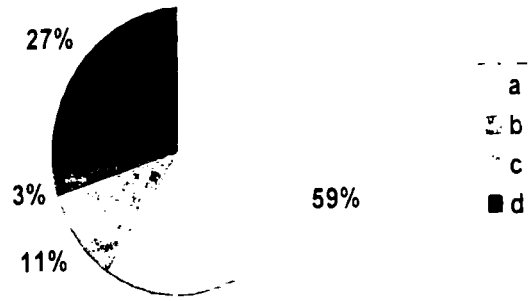

Figure 4. Answers to question no. 6

Legend for figure no. 4

If you followed a diet to cure a disease, did you also undergo a treatment (medical, surgical, x-ray) recommended by the doctor?
a. yes
b. no, but I followed a medical treatment until starting the diet
c. no, but I followed a medical treatment after finishing the diet
d. no, I didn't, before or after the diet

forms, was considered the most efficient in curing diseases by 53.8% of the subjects.

3. The answers to the third question showed that when people are going on a diet in order to cure a disease, 86% of the respondents think that patients should undergo in the same time the treatment recommended by a doctor; 13.8% consider that patients should accept no conventional, that is, pharmacological, surgical or X ray treatment (Figure 3).

4. Data gathered from answers to question no. 4 indicated that 25% of the people participating in the survey have not met persons who were cured from a serious or incurable disease by following a diet; 62.5% have heard of these cases by word of mouth, media, Internet and 37,5% have personally met such persons.

5. The answers to question no. 5 showed that 79.3% of the respondents went on a diet, for various reasons. A proportion of 38.2% were motivated by the desire to preserve their health, 30% for weight loss, and 24.4% for detoxification. Also, 22.70% of them went on a diet with the intent to treat a disease and 4% tried a diet with the purpose to control an incurable disease.

6. According to the answers to question no. 6, out of those respondents who tried a therapeutic diet, 73.1% followed a conventional treatment, simultaneously, before or after the diet. On the other hand, 26.9% of these subjects did not follow the conventional treatment prescribed by the specialist, neither before, nor after the dietetic cure. Most of these were young people under 30 years of age, from rural residency and medium level of education. (Figure 4)

7. The answers to question no. 7 indicated that in 40% of the cases, the decision to start a therapeutic diet is a personal one.

8. The answers to question no. 8 brought out that the main reason behind a food cure is the preference for natural remedies in 45.2% of the cases, the religious believes in 10.6% of the cases, the fear of adverse effects of the conventional treatments in 6.1% of the cases, being uncertain about the tratment recomanded by the physician in 2,6% of the cases, and the inefficiency of conventional treatment in 1.3% of the cases. 34.2% of the subjects had other unspecified reasons.

9. According to the answers to the last question, the diet was complemented by various remedies and procedures as follows: drinking more water (67.7%), physical exercise (58.0%), herbal teas (52.5%), prayer (36.1%), food suplements (32.9%). Other complementary procedures aplied were: fasting, relaxation exercises, meditation, massage, homeopathy etc.

DISCUTIONS AND CONCLUSIONS

1. A high proportion of the Romanian Internet users express total confidence in the preventive and curative virtues of diets. Almost 40% of the respondents consider that nutrition (especially vegetarian diets) can prevent and cure all diseases.

2. Most of the subjects surveyed (86%) consider that in case of experimenting a therapeutic diet, the pacient should undergo in the same time the conventional treatment recomanded by the medical

doctor. A small but still allarming proportion of the participants in the survey (13%) consider that the pacients should not accept any conventional treatment at all.

3. Most of the subjects (~80%) who have total confidence in the preventive and curative properties of food, think that when starting a food cure, the pacient should still receive conventional treatment under the physician's direction, while ~20% of them believe that the pacient should not accept any kind of conventional medical procedure.

4. An important proportion of the population studied (over 20%) tried at least one kind of therapeutical diet. In ~5% of these cases, the diet was introduced with the purpose of treating an incurable disease like cancer. The high esteem for the so called natural treatments or remedies plays an important rol in almost half (45.2%) of the subjects who experimented a therapeutical diet. Another important motivation that influenced the treatment options in our study was the religious belief.

5. Almost 25% of those who went sometime on a therapeutical diet, din not follow the treatment recomanded by the physician, neither before, nor after the unconventional therapy. This exclusivistic atitudine represents a serious risk in pacients suffering from diseases in which delaying the medication or surgical operation is critically important for healing or survival. More than a third (35.8%) of those believing that nutrition can prevent and cure all the diseases, did not undergo a conventional therapy while they started a diet with the purpose of treating a disease.

6. Although can not be extrapolated to the general population, the results of our survey sugests that many people could make critical decisions about treatment options based on scientifically unsustained believs about the preventive and curative properties of food in general and certain diets. Our study shows that there is a need to better inform people about the real benefits of healthy nutrition.

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Hepatitis B Virus and Human Immunodefficiency Virus Coinfection – evolutive aspects (preliminary results)

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Hypothesis: in this study we proposed to answer the question: there is any influence of HBV infection on the natural hystory of HIV infection? Methods: This study is a retrospective cohort of HIV infected patients, B2 category in 2001, taking TARV at least from 2001 which were followed up for 7 years. We noted medical recording concerning clinical events between january 2001-decembre 2007 in selected patients. Results: 54 patients were enroled, 22 with HIV-VHB coinfection and 32 with HIV infection. Deaths occured in 9% of HIV-HBV coinfected patients and in 3,12% of HIV infected patients (p>0.05). 20.4% of patients developed an AIDS-associated clinical events, 18,8% of HIV infected patients, vs 21,87% of HIV-HBV coinfected patients (p>0,05). At the end of follow up period, CD_4 lymphocytes count and HIV viral load were similar in both lots: 402±246,33 cels/mm³ in HIV-HBV coinfected vs 404,5±307,04 cels/mm³ in HIV infected (p>0,05), respectively, 3,63±0,91 log_{10} in HIV-HBV coinfected, vs 3,77±1,04 log_{10} in HIV infected (p>0,05%). Conclusions: There were no significant differences between the two lots in number of deaths, AIDS-associated events, CD_4 lymphocytes and HIV viral load.

***Key words**: HIV-VHB coinfected, HIV infected*

Since the introduction of HAART mortality and morbidity of HIV infected patients have decreased while the life-span increased, thus the management of chronic HIV-associated condition (other than HIV infection and opportunistic determinations) becomes more important.

HIV and HBV is frequently associated, because of shared routes of transmission; some authors claims that 80-90% of HIV infected individuals have at least one serological evidence of previous exposure to HBV[7,9], while chronic hepatitis B is seen in 10% of HIV infected patients.[2,3,4,7,9]

A recent prospective study – EuroSIDA cohort – did not find evidences of HBV impact on HIV evolution or HAART response.[3] Another study – HIV-NAT cohort – reaches the same conclusion, but noted a delayed CD4 increment among HBV-HIV coinfected subjects following HAART.[5]

There are authors claiming that HBV influences the evolution of HIV infection as following: HIV replication is increased[7,11], HAART associated hepatotoxicity is incremented[1,7,10,11], CD4 count is notable lower in HIV infected subjects diagnosed with cirrhosis and splenic sequestrations of blood cells[8], HBV itself decreases the CD4 count.[7]

Globally data reffering to HIV-HBV coinfection and the impact on human host are conflicting.

Infectious Disease Department, Faculty of Medicine, University of Medicine and Pharmacy of Craiova

METHODS

It is a retrospective study; we have analysed a cohort oh HIV infected patients under surveillance of Regional Center for Monitoring and Surveillance of HIV/AIDS infection – Craiova, B2 classification (CDC, Atlanta, 19XX) in 2001, taking antiretroviral treatment (ART) at least since 2001, wich were followed up for the next 7 years.

They have been grouped into two categories:

-HIV-HBV coinfected patients having at least two HBs antigens determinations, six month apart one to another

-HIV infected patients with negative HBs antigen determination

We have analysed: eath rate, frequency of associated clinical events, evolution of the CD4 count and HIV viral load; comparation between the two groups were based on Student t test (statistical singnificance being characterized by $t < 0.05$).

RESULTS

Descriptive data

Of the 54 patients enrolled in our study, 22 were HIV-HBV coinfected, with the median age of 12±0,67 years, sex ratio M/F=9/13, all of them being exposed to HIV by parenteral way. The 32 infected HIV patients had a median age of 12±7,77 years, sex ratio M/F=21/11, 31 being exposed to HIV by parenteral way and 1 by sexual way. No difference was observed in CD_4 cell count at enrolment either at the end of following up

between coinfected HIV-VHB patients and HIV infected patients (342±135,18 cells/mm³ in coinfected vs 474±166,54, in monoinfected, p>0,05, respectively 402±246,33 cells/mm³ in HIV-HBV coinfected vs 404,5±307,04 i HIV monoinfected, p>0,05). HIV viral load did not differ between HIV-HBV coinfected and HIV infected patients at enrolment either at the end of following up (4,1±0,96 $\log_{10}$copies/ml in HIV-VHB coinfected, vs 4,4±1,06 $\log_{10}$copies/ml in HIV monoinfected, p>0,05, respectively 3,64± 0,91 $\log_{10}$ copies/ml in coinfected vs 3,77± 1,04 $\log_{10}$ copies/ml in monoinfected, p>0,05) (Table I)

Clinical events

Figure 1 and 2 illustrate the incidence of clinical events in the two lots on the following up period.

During the study period 3 patients died (5,5%). No difference was seen in incidence of mortality between the two lots; 2 deaths occurred in HIV-HBV lot (9,1%) and 1 death occurred in HIV lot (3,12%) (p>0,05). All deaths have been caused by AIDS-associated conditions.

11 patients (20,4%) developed AIDS-defining clinical events on the following up period, 4 of them were HIV-HBV coinfected and 7 were HIV infected (P>0,05)

Table I. Demographic characteristics of the patients according to hepatitis B status

	HIV-VHB coinfected	HIV monoinfected	p
Age(median)	12 (SD 0,67)	12 (SD 6,77)	
Male	9	21	
Female	13	11	
Parenteral route	22	31	
Sexual route	0	1	
CD4 at enrolement -median, SD	342 (SD 135,18)	474 (SD 166,54)	>0,05
CD4 at the end of following up -median, SD	402 (SD 246,33)	404,5 (SD 307,04)	>0,05
VL at enrolement -median, SD ($\log_{10}$ copies/ml)	4,1 (SD 0,96)	4,4 (SD 1,06)	>0,05
VL at the end of following up -median, SD ($\log_{10}$ copies/ml)	3,64 (SD 0,91)	3,77 (1,04)	>0,05

Table II. Clinical events of the patients according to hepatitis B status

Clinical events	HIV+VHB	HIV	p
Deaths	2 (9%)	1 (3,12%)	>0,05
AIDS-deffining clinical events	4 (18,18%)	7(21,87%)	p>0,05

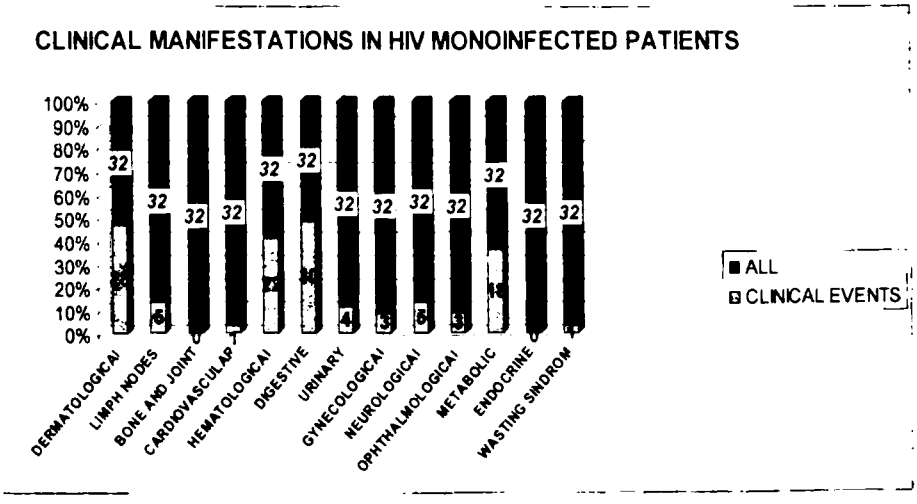

Figure 1. Clinical manifestations in HIV monoinfected patients

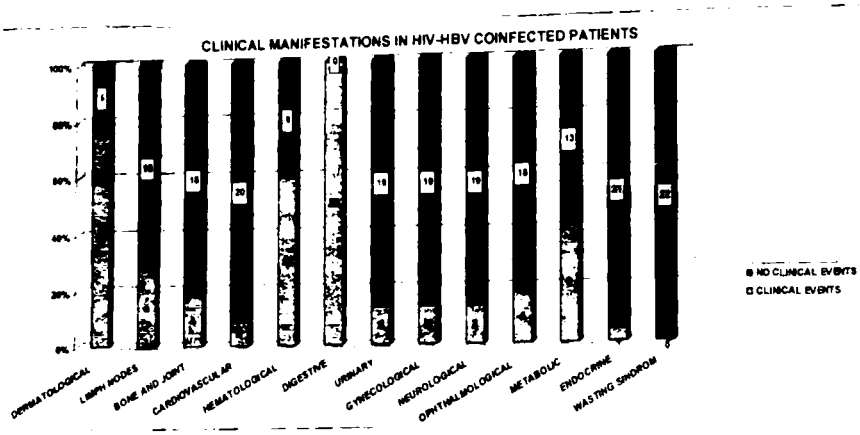

Figure 2. Clinical manifestations in HIV-HBV coinfected patients

DISCUSSIONS

EuroSIDA cohort analysis (72 european centers, 5728 HIV infected patients included from wich 498 coinfected with HBV, followed-up 8 years) denoted an increased risk of death among coinfected subjects (RR=1.53, 1.23-1.9, p=0.0009)[3]. However the median duration of HIV infection was longer in coinfected patients (5.3 vs 4.2 years, p<0.0001)[3]; also at the baseline the CD4 count were significantly lower and the HIV viral load were higher among HIV-HBV coinfected, so this might be the cause of the greater rate of death[3] Sexual transmission for HIV and HBV were mostly recognized in EuroSIDA cohort[3]. Ag HBs status did not influence viral or immunological responses to HAART among EuroSIDA cohort [3].

HIV-NAT cohort analysis (692 HIV infected patients included from which 60 coinfected with HBV, followed-up 48 weeks) did not reveal an increased risk of progression to AIDS-defining events or deaths among patients coinfected with HIV and HBV compared with HIV infected patients, but the authors noted that a longer follow-up is required to further assess the impact of HBV hepatitis coinfection status on HIV disease progression, liver disease-related and overall mortality[5]. The majority of patients in HIV-NAT cohort were infected (HIV, HIV-HBV) by parenteral route. Median HIV RNA reduction was similar in HIV infected and HIV-HBV coinfected patients. HIV patients with HIV-HBV coinfection had an early, but not sustained CD4 cell recovery.[5]

Our study noted a differences concerning death rate, but not with a statistical significance. Apart from EuroSIDA cohort, our research highlighted the resemblance between the two groups regarding the length of HIV infection. It was also noted that in our case unlike Euro-SIDA and NAT-HIV cohorts, parenteral transmission, during childhood, of both HIV and HBV were dominant. All this facts might have an impact on death rate.

It is also well known that different HIV and HBV subtypes, with different geographic distribution, have distinct clinical and evolutive patern of evolution and maybe this issue is also important for the appreciation of evolution of HIV-HBV coinfection in a study.

There were no statistical differences between the two groups in term of incidence for AIDS-defining clinical events both in our study as well as in EuroSIDA cohort.

As other authors have noted before[3,5], no statistical significant differences were noted between CD4 cell recovery and HIV RNA reduction in HIV infected and HIV-HBV coinfected in our study

CONCLUSION

There was no significant differences between the two groups concerning death rate, AIDS associated-

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Testicular germ cell tumors - a retrospective study

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Seminomatous and non-seminomatous testicular germ tumors are rare but however remain the most common testicular tumors. Histological typing is essential in order to establish therapeutic conduct. The study aimed to establish the frequency of different histological types of testicular germ tumors and the correlation with the patient's age. The study was performed in the Surgical Pathology Department of the Emergency County Hospital, Târgu Mures and has analyzed the cases of testicular germ cell tumors between January 2004 and December 2008. We used the WHO 2004 histological classification in determining the type and the UICC 2002 classification in order to establish the tumor stage. 59 testicular germ cell tumors were diagnosed during this period. 16 of them were pure seminomas. Most patients with seminomatous tumors were in the 4th decade of life, with an average age of 39 years, and those with non-seminomatous tumors were in the 3rd decade, with an average of 29 years. Embryonal carcinoma was the most frequent histological type. Yolk sac tumor was characterized by a high architectural variability and choriocarcinoma was very rare. Imunohistochemistry was used in 18 cases, especially in mixed germ cell tumors.

Key words: *testicular germ cell tumors, frequency, immunohistochemistry*

Testicular germ cell tumors are rare, but still represent the majority of malignancies of the testis. Treatment and prognosis divide them into two main categories: seminomatous and non-seminomatous tumors. Any non-seminomatous elements (even if seminoma is prevalent) exclude the diagnosis of seminoma.

For unexplained reasons there is a worldwide increase in the incidence of germ cell tumors, with an increasing number of mixed germ cell tumors in the detriment of seminomas. They affect young patients, between 15 and 35 years of age, with rare prepubertar cases. Incipient stage tumors are treatable, with a 5 years survival rate of almost 100%.[2,3,4,6]

MATERIAL AND METHODS

The aim of this study is to evaluate retrospectively the distribution of testicular germ cell tumors in a 5 years population. The study was performed in the Department of Surgical Pathology of the Emergency County Hospital, Târgu Mures and included all cases of testicular germ cell tumors diagnosed between January 2004 and December 2008. All clinical data were obtained from the patients' records. For the pathological diagnosis all the hematoxylin-eosin stained slides were reviewed. Tumors were classified as seminomatous or non-seminomatous according to their microscopic characteristics established by the 2004 WHO classification of testicular tumors. The TNM stage was stated using the UICC classification published in 2002.

Immunohistochemistry was used in 18 cases. Antibodies against cytokeratin, AFP, CD30, PLAP and HCG were used for the diagnosis of retroperitoneal metastases, in appreciating different components of mixed germ cell tumors, in difficult cases with a complex architecture and for the differential diagnosis of seminomatous and non-seminomatous, pure or mixed tumors. We used monoclonal and polyclonal antibodies from Dako and Novocastra and the immunohistochemistry was performed using a three-step indirect process based on the streptavidin-biotinylated peroxidase complex method.

Because no data about patient evolution were available, we could not appreciate their outcome.

RESULTS

Of the 59 testicular tumors diagnosed, 16 cases were pure seminomas, representing 27.11% of all tumors. 41 were non-seminomatous tumors, with 2 cases at a prepubertar age. 31 cases were mixed germ cell tumors, of which the association between embryonal carcinoma, yolk sac tumor and teratoma was the most frequent. The ratio between non-seminomatous and seminomatous tumors was almost constant, in favor of non-seminomatous tumors.

16 cases of pure seminoma were evaluated. Seminoma was associated with other histological contingents in only 9 cases.

Embryonal carcinoma was the most common histological type, appearing as a component in 24 of all

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Table I. Case distribution according to the histological type

	seminoma	embrional carcinoma	Yolk sac tumor	choriocarcinoma	teratoma
Pure tosticular germ cell tumors	16	4	4	1	4
Mixed testicular germ cell tumors	9	24	21	4	21

Table II. Case distribution according to the patient's age

age / histologic type	0-10	11-20	21-30	31-40	41-50	51-60
Seminomas	0	0	1	8	5	2
Non-seminomatous testicular germ cell tumors	2	8	18	12	2	1

mixed germ cell tumors reviewed. It was rarely found as a pure tumor (4 cases).

Most cases of the yolk sac tumors were diagnosed after puberty (25); only 1 pure case was evaluated at a prepubertar age.

A single case of pure choriocarcinoma was identified. We also found 4 cases of mixed germ cell tumors containing choriocarcinomatous contingents.

21 teratomatous contingents were part of a mixed germ cell tumors and only 4 cases were diagnosed as pure teratomas, 1 of which at a prepubertar age. Two teratomas had areas of somatic type malignancies. One case of epidermoid cyst was seen in association with seminoma (Table I).

Regarding the age distribution, we noticed that the cases were generally diagnosed in the 3rd and 4th decade of life. Most patients with the diagnosis of seminoma were in the 4th decade of life, with a medium age of 39 years. The majority of patients with non seminomatous tumors were in the 3rd decade with a medium age of 29 years. Assessing the patient's age with mixed germ cell tumors which contained a seminomatous contingent, we obtained a mean age of 31 (Table II).

48 tumors were diagnosed as pT1 stage, 7 as pT2 and 4 as pT3. No cases of pT4 tumors were found.

In 52 cases, the testicular peritumoral parenchyma presented intratubular germ cell neoplasia unclassified. Focal aspects of intratubular embryonal carcinoma were observed in only 1 case. Prepubertar cases were not associated with intratubular germinal neoplasia.

Immunohistochemistry of seminoma was characterized by a membranous positivity for PLAP and cytokeratin negativity. All non-seminomatous tumors were cytokeratin positive. Embryonal carcinoma was CD30 positive; yolk sac tumor was AFP positive and the syncytiotrophoblasts of choriocarcinoma and those of seminoma, were assessed with anti-HCG antibodies.

DISCUSSION

Testicular germ cell tumors spam a large spectrum of malignant entities, representing 1-2% of all the malignant tumors involving young male patients.[1,5,6,7]

In our study, most seminomas were found in the 4th decade, whereas non-seminomatous cases were seen in the 3rd decade of life. The average age of patients with mixed germ cell tumors was 10 years below that of seminomatous cases. Similar findings were reported by other studies. Mixed tumors with a seminomatous contingent developed with a delay of 3 years, which could mean that seminoma has a slower pace of development than other testicular germ cell tumors. This is why, besides the classic development of germ cell tumors of the testis from a malignant cell (intratubular germ cell neoplasia with an intermediate stage of seminoma) we can imagine other pathways of development. One of them might be direct development from differentiated cases of intratubular germ cell neoplasia. However, this theory is difficult to demonstrate due to the rarity of differentiated cases of intratubular germ cell neoplasia.

In the international reports seminoma accounts for approximately 50% of all germinal cell tumors of the testis.[1,4,5,6,7] For unexplained reasons a decreasing trend of these cases was also noted. Very interestingly, the results of our study showed that seminoma represents only 27% of all tumors, which is not in accordance with others' data. These results are probably influenced by potential geographical and social factors. 2 cases were associated with syncytiotrophoblasts that sometimes might cause an elevated serum level of bHCG, but not as elevated as in choriocarcinoma. Cell pleomorphism was observed in only two cases. This variant of seminoma, recognized by WHO classification, is not accepted by most of the authors especially because it has the same prognosis as the classical one.[1,5,7] Seminomas have very typical morphological aspects and usually the diagnosis doesn't require imunhistochemical examination. However immunohistochemistry was carried out in 2 pure seminomas with unusual tumor architecture, yielding a membranous positivity for PLAP and negativity for cytokeratin. Immunohistochemistry was also used in 2 cases of seminomatous retroperitoneal lymph nodes metastasis.

In our cases embryonal carcinoma was the most common histological type, identified in almost 47% of all non-seminomatous tumors. We also noticed that the cases of pure embryonal carcinoma were rare. Embryonal carcinoma was most frequently associated with yolk sac tumor and teratomatous contingents, these cases representing about 25% of all mixed germ

cell tumors reviewed. Embryonal carcinoma has many morphological features in common with yolk sac tumor, and this is why immunohistochemistry is very important for the differential diagnosis, especially that embryonal carcinoma is more aggressive than yolk sac tumor. Embryonal carcinoma cells presented a membranous positivity for CD30.[1,5,7]

Yolk sac tumor is an impressive and very spectacular tumor, characterized by a great variety of architectural patterns. It expresses antibodies against cytokeratin and AFP. All cases were cytokeratin positive, with a membranous pattern. AFP presented focal cytoplasmatic positivity, which corroborated with cyto-architectural aspects allowed us a correct diagnosis.[3]

In our study we found that choriocarcinoma is very rare, even exceptional as a pure tumor (1 case). Very often these tumors have already lung hematogenous metastases at the time of presentation. The presence of syncytiotrophoblasts, cytotrophoblasts and intermediate trophoblasts which are trying to reproduce the normal placental villi is necessary for the diagnosis of choriocarcinoma. In identifying syncytiotrophoblasts we used antibodies against HCG.

Generally, teratomas are easily identified and according to WHO 2004 testicular germ cell tumors classification, we made no distinction between mature and immature morphologies. Teratomatous elements in a lymph node must be very carefully appreciated because they can mimic visceral metastases with origin other than testicular.[1,5,7]

CONCLUSIONS

Testicular germ cell tumors are rare, but they still represent the majority of the testicular malignancies.

In our study non-seminomatous tumors represented the majority of the cases, with a greater frequency than in other studies, but respecting the actual upward trend of mixed testicular germ cell tumors.

Most seminomas were diagnosed in the 4th decade of life and non-seminomatous tumors in the 3rd decade. A difference of 10 years between these groups of tumors was also observed.

Prepubescent cases were not associated with intratubular germ cell neoplasia.

The majority of tumors had a low stage, which is a very important and a favorable prognosis factor.

Immunohistochemistry had an essential role in establishing the pure character of a seminomatous testicular tumor or for precise identification of other histological types in a mixed germ cell tumor.

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The histological characteristics of placenta

Mihaela Viorica Stanculescu[1], Maria Sajin[1], Maria Comanescu[2], Gabriela Bruma[1]

Placenta is a transitory organ which assures the nutrition, growth and development of the fetus. The aim of this communication is to show the histological study of normal placenta depending on the gestational age. Material and method: a clinical-morphological study was performed on a retrospective lot of 159 cases, analysed in 2008 at the University Emergency Hospital, Bucharest. The fragments were fixed in formaldehyde 10%, embedded in paraffin, cut and stained with Hematoxylin-Eosin and Van Gieson. Results and discussion: from all 159 cases, 117 cases are placentas and 42 cases are fragments of placenta from the uterine curettages. We analysed macroscopically all placentas depending on their sizes and the aspects of the all chorionic villi. Conclusions: this study is wanted to be a guide-diagnosis. The histology of placenta is good to be known, because in this way we can differentiate the pathological cases from the normal cases

***Key words**: pregnancy, gestational age, placenta, villi*

Placenta is a transitory organ which assures the nutrition, growth and development of the conceptus and at the same time, it protects it from the substances which run in the maternal blood through the maternal-fetal barrier. The components of placenta are formed in the first days of pregnancy. Placenta is an unique organ. It is the only organ formed of the tissues that come from two varied organisms: maternal and fetal. In normal conditions, the placenta is completely eliminated after birth.[4]

The aim of this communication is to show the histological study of normal placenta depending on the gestational age.

MATERIAL AND METHODS

The biological material used is represented by the fragments of placenta and placentas from the Obstetrics-Gynaecology and Pathology Departments of The University Emergency Hospital, Bucharest. The clinical-morphological study is effectuated on a retrospective lot of 159 cases, which were analysed in 2008. After the macroscopic exam, the fragments were fixed in formaldehyde 10%, embedded in paraffin, cut and stained with Hematoxylin-Eosin and Van Gieson.

RESULTS AND DISCUSSION

From all 159 cases, 117 cases are placentas and 42 cases are fragments of placenta from the uterine curettages. 43 cases come from the pregnancies in the first term, 10 cases from the second term and 106 cases from the third term. Most women are in the second decade of life, 88 cases; 66 women are in the third decade of age, 4 women are in the fourth decade of age and only a woman is 17 years old.

Macroscopic: at term, placenta is discoid, flat, round or oval. It is formed from a fetal section (chorion) and a maternal section (deciduas basalis).[3] The diameter of the term placenta is approximate 22 cm, the thickness is 2,5 cm and the weight is 470 g. The umbilical cord is white-pearled and has approximate 55 cm length and 1-1,5 cm in diameter. Frequently, it is eccentrically inserted and usually, it contains 3 vessels: 2 arteries and one vein. It can have a marginal or velamentous insertion. Occasional, it can appear only an artery or a second persistent vein. The fetal membranes are translucent and radiant.[6,7] In our study, from all 117 cases of placenta, 60 placentas were 8-22 cm in diameter, 35 placentas were 15-18 cm in diameter and 22 cases were fragmentary placentas, 10-15 cm. 90 placentas had a central insertion of the umbilical cord and 27 placentas had a marginal insertion of the umbilical cord. The length of the umbilical cord alternated between the 25-55 cm. The functional units of placenta are the chorionic villi, which are form through the proliferation of the cytotrophoblast cells.

In the first term of the pregnancy, the villi are formed from a syncytiotrophoblast layer at the periphery and a cytotrophoblast layer in the inner zone. Later on, in the second part of pregnancy, the cytotrophoblast layer becomes atrophic and the syncytiotrophoblast layer persists until birth. The villi covered by the syncytiotrophoblast interpenetrate with the decidua basalis, between them are persisting the lacunae of the trophoblast filled with maternal blood.[6] Depending on their morphology, the chorionic villi are 3 types:

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- the primary chorionic villi: start to form in a 11-13 days of pregnancy. They are the smallest villi, without extensive ramifications. They are formed from the proliferation of the cytotrophoblastic cells, in the shape of columns which invade the trabeculae of syncytiotrophoblast and intermediate trophoblast between the sanguine lacunae. The primary villi grow out to the periphery and spread out over the interface between the trophoblast and endometrium forming the cytotrophoblast shell.[1,4]

- the secondary chorionic villi: have ramifications and they are formed when the extraembryonic mesoderm invades the primary villi, which thus develop a mesenchymal core.[1,4]

-the tertiary villi: have very long ramifications and they are developed from the secondary chorionic villi when the mesenchymal cores of the villi become vascularised, in the end of the third pregnancy week.[1,4]

The villi are formed on the whole circumference of the chorion until 8 week. After that, they persist and grow only the villi that, there are in contact with the decidua basalis. The rest of them degenerate and the surface is smooth and slight vascularized. The extensive ramifications of the villi achieve a big exchange surface for the nutritive substances approximate 10 mp. And the surface is more enlarged by the microvilli of the syncytiotrophoblast until 90 mp.[4]

The ramifications of the villous trees can be subdivided into 5 villous types based on caliber, stroma, vasculature, position within the villous tree and development: mesenchymal villi, immature intermediate villi, mature intermediate villi, stem villi and terminal villi.[5]

In early placenta are mesenchymal and immature intermediate villi and in the term placenta are all 5 types of villi.

In our study, we identified in all 43 cases from early placenta (8-12 weeks), the mesenchymal villi and immature intermediate villi. The mesenchymal villi were invested by a thick trophoblast, comprising of an inner layer of cytotrophoblast cells (Langhans' cells) and a broader outer syncytiotrophoblast layer.[5] We also identified some areas with the intermediate trophoblast. The fetal capillaries were poorly developed (Figure 1).

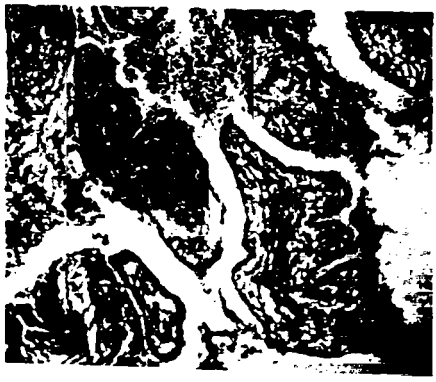

Figure 1. The mesenchymal villi, (HE x 100)

The immature intermediate villi were bulbous in shape with a thick trophoblastic cover, formed from syncytiotrophoblast and proeminent Langhans'cells. There was a reticular stroma with fluid-filled stromal channels and Hofbauer cells[2,5] (Figure 2).

Figure 2. The immature intermediate villi, (HE x 40)

We identified the mesenchymal villi in 65% and immature intermediate villi in 53% .

In the 106 cases from term placenta we identified the mesenchymal villi and also the stem villi, mature intermediate villi and terminal villi.

The stem villi, also called anchoring villi, had a variable caliber. In the mature placenta, their surfaces were often degenerative and partially replaced by fibrinoid. They had a thin layer of syncytiotrophoblast only and the stroma consisted of condensed bundles of collagen fibers with occasional fibroblast and rare macrophages Most villi had a central artery and the capillaries tended to be located in the periphery of the core[5] (Figure 3).

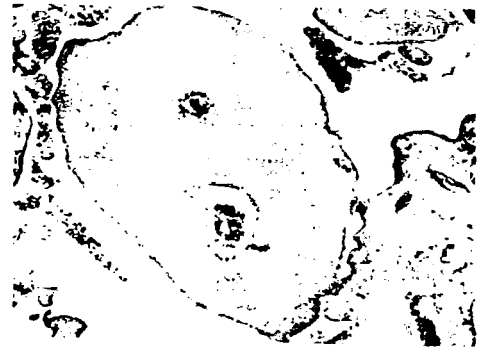

Figure 3. The stem villi, (HE x 100)

The mature intermediate villi were long and slender. They had only a syncytiotrophoblast layer and the stroma consisted of seemingly unoriented, loose bundles of connective tissue and connective tissue cells and numerous capillaries, arterioles and venules[5] (Figure 4).

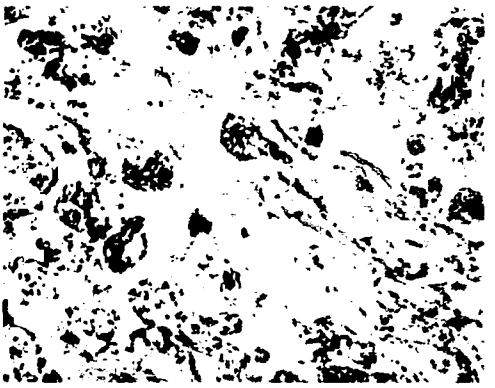

Figure 4. The mature intermediate villi, (HE x 100)

The terminal villi were grapelike outgrowths of the mature intermediate villi and appeared as single villi or a poorly ramified side branches.They had a thin trophoblastic cover in intimate contact with the capillaries. Stroma consisted of scant connective tissue fibers and rare macrophages.[2,5] (Figure 5).

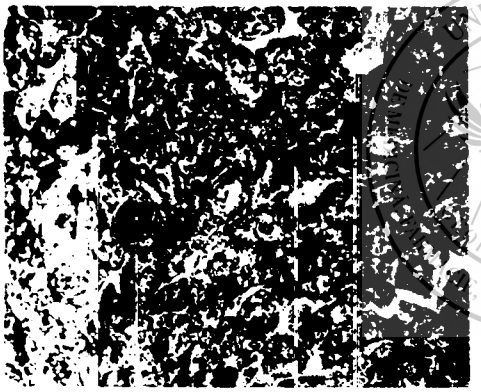

Figure 5. The terminal villi and syncytial knots, (HE x 200)

We found prevalent terminal villi 55%, the mature intermediate villi 43% and stem villi 2%. We also identified the syncytial knots. Their syncytiotrophoblast nuclei are aggregated together in clusters. These syncytial knots are a feature of the term placenta.[1]

CONCLUSIONS

This study is wanted to be a guide-diagnosis. The histology of placenta is good to be known, because in this way, we can differentiate the pathological cases from the normal cases.

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Papillary thyroid microcarcinomas associated with autoimmune thyroid diseases

Zsuzsanna Szántó[1], I.Z. Kun[1], Angela Borda[2], J. Jung[2], J. Bécsi[1]

We studied the association of thyroid microcarcinomas with Hashimoto's thyroiditis or Graves' disease, and we appreciated the frequency of microcarcinomas with a potential transformation into clinically overt thyroid cancer. Material and methods. We included in the study patients diagnosed with uni-/multinodular goiter and/or autoimmune thyroid disease at the Endocrinology Clinic Târgu Mures who were diagnosed histologically with papillary thyroid microcarcinoma during 2005-2008. The 89 papillary thyroid microcarcinomas were divided into two groups: 64 without and 25 associated with autoimmune thyroid disease. Results. Autoimmune thyroid diseases were associated significantly more frequently with papillary thyroid microcarcinomas (28%) than with clinically overt thyroid cancers (14%) (p=0.028, 95%CI:1.1–3.7). Among papillary thyroid microcarcinomas without autoimmune thyroid disease 17.2%, and from cases with autoimmune thyroid process 4% were in stage III. Conclusions. The higher rate of occult tumor in patients with papillary thyroid carcinoma and Hashimoto's thyroiditis as well as the lower rate of microcarcinomas in advanced TNM-stage coexisting with autoimmune thyroid disease suggest that the autoimmune thyroid process is a defence reaction against malignant thyroid lesion.

Key words: papillary thyroid microcarcinoma, Hashi-moto's thyroiditis, Graves' disease

In recent years the incidence of papillary thyroid microcarcinomas increased worldwide, partly due to the improvement of diagnostic tools used in thyroidology (e.g. high resolution neck US). Thyroid microcarcinomas are present in about 30% of thyroid samples obtained by step section at autopsy and in up to 24% of thyroids removed for other reasons.[12,18] Most of these microtumors are without any clinical significance. While most pose no threat to survival and require no further surgery[13], about 20% are multifocal and as many as 60% have cervical lymph node metastases.[2] Consequently, microcarcinomas associated with metastases should be treated in the same way as larger lesions[12], so the debate about the treatment continues. Microcarcinomas with multifocal spread, extrathyroidal extension and/or lymph node metastases harbour a higher risk to tranform into clinically overt thyroid carcinomas.

The coexistence of Hashimoto's thyroiditis and papillary thyroid carcinoma is controversial. Some authors suggest that Hashimoto's thyroiditis is a precancerous state, but according to others the immune response might be important in preventing metastasis and recurrence of thyroid cancer.[1,3,6,7,8,9,11,17,19,22] Patients with autoimmune thyroiditis and papillary thyroid carcinoma generally have improved disease-free survival 35 years after primary surgery.[14]

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Our objectives were to study the coexistence of thyroid microcarcinomas with Hashimoto's thyroiditis or with Graves' disease, and to appreciate the frequency of microcarcinomas with a potential transformation into clinically overt thyroid cancer, studing histological presentation, TNM-staging of papillary thyroid microcarcinomas associated or not with autoimmune thyroid diseases.

MATERIAL AND METHODS

We included in the study patients with uni-/multinodular goiter and/or autoimmune thyroid disease diagnosed at Endocrinology Clinic Târgu Mures, who underwent thyroidectomy during 2005-2008. The tissue specimens were evaluated at the Institute of Pathology Târgu Mures. The diagnosis of Hashimoto's thyroiditis, Graves' disease and goiter was established by common clinical, laboratory (antithyroid antibodies) and imagistic criteria (thyroid ultrasound), and the final diagnosis of thyroid malignancy was established by histological exam. In case of microcarcinomas (< 15 mm) we took into account the size, histological variant of papillary carcinoma, uni-/multifocality, bilateral spread, extrathyroidal extension, lymph node metastases and TNM-stage. The 89 papillary thyroid microcarcinomas were included in two groups: 64 microcarcinomas alone, and 25 Hashimoto's thyroiditis (and/or Graves' disease) coexisting with papillary thyroid microcarcinoma.

RESULTS AND DISCUSSION

Among patients diagnosed with uni-/multinodular goiter and/or autoimmune thyroid disease who underwent thyroidectomy during 2005-2008, a number of 223 thyroid cancers were detected. All thyroid microcarcinomas were papillary histotypes. From the 182 papillary thyroid cancers 89 (49%) were microcarcinomas, and in this latter group 25 were associated with autoimmune thyroid process.

Table I shows that autoimmune thyroid diseases were associated significantly more frequently with papillary thyroid microcarcinomas (28%) than with clinically overt thyroid cancers (14%) (p=0.028; 95%CI: 1.10–3.67), and Hashimoto's thyroiditis dominated versus Graves' disease. These data are similar with results in literature.[8] Other studies reported that Hashimoto's thyroiditis coexists more frequently with well-differentiated thyroid carcinoma.[9] The higher rate of occult tumor in patients with papillary thyroid carcinoma and Hashimoto's thyroiditis than in cases with papillary thyroid carcinoma alone, may have two explanations: 1. the autoimmune thyroid disease is a defence reaction against malignant thyroid nodules, and/or 2. Hashimoto's thyroiditis is a common thyroid disease, it appears as a large nodular goiter, so patients suffering from it may reach the specialist sooner than patients having ocult thyroid carcinoma alone.

In our casuistic the mean age of the 64 patients with papillary thyroid microcarcinoma alone (50.5±13.3 years) was significantly higher than that of the 25 patients diagnosed with autoimmune thyroiditis and microcarcinoma (43.9±10.7 years) (p=0.027). The rate of patients younger than 45 years was 29.7% in the first group and 52% in the second group. In the literature most patients with papillary thyroid carcinoma coexisting with Hashimoto's thyroiditis are female and younger than 40 years.[8] Immune response depends on age, younger patients have a well functioning immune system, therefore evolution and prognosis of thyroid cancer is better than in old people.

Thyroid microcarcinomas are usually without any clinical significance, and mostly they are detected incidentally by histology or autopsy. In order to detect papillary thyroid microcarcinomas which might evoluate toward clinically overt carcinomas, we followed the uni-/multifocality, extrathyroidal extension, aggressive histological variant, regional lymph node metastases, in the presence/absence of autoimmune thyroid disease.

Papillary thyroid microcarcinomas without autoimmune thyroid process were multifocal in 14 cases (23.4%): 3/4 showed microfoci in both thyroid lobes, 1/4 multiple microfoci spread in a single lobe. Papillary thyroid microcarcinomas with autoimmune thyroid disease were multifocal in 19% (4 cases: 3 bilateral and 1 unilateral) (p=0.57).

Papillary thyroid microcarcinomas without autoimmune thyroiditis showed extrathyroidal invasion in 14% of cases versus 12.5% of patients having both diseases.

The diffuse sclerosing, solide/trabecular, collumnar-cell and tall-cell variants of papillary thyroid cancer have an aggressive course, so we focused on these variants. Among all 89 microcarcinomas 6 (6.7%) had a potential aggresive course: 5 (3 trabecular, 2 tall-cell variants) without autoimmune thyroid process (7.8% out of 64), and one trabecular tumor associated with Hashimoto's thyroiditis (4% out of 25).

6 (6.7%) papillary thyroid microcarcinomas presented lymph node metastasis: 4 patients (6.3%) without and 2 (8%) with autoimmune thyroid disease.

The presence of one or more of the four studied parameters (multifocality, extrathyroidal extension, aggressive histologic variant, metastases in lymph nodes) in microcarcinoma suggest a possible transformation into clinically overt thyroid cancer: we found 33 patient (37%) having with these parameters (Table II).

Table I. The frequency of autoimmune thyroid diseases in clinically overt thyroid cancer and thyroid microcarcinomas

Autoimmune thyroid process	Clinically overt papillary thyroid cancer (diameter > 15 mm)			Papillary thyroid micro-carcinoma (diameter < 15 mm)		
Hashimoto's thyroiditis	11	12%	14%	20	22.5%	28%
Graves'disease	1	1%		5	5.5%	
Hashitoxicosis	1	1%		0	0%	
Without autoimmune thyroid disease	80		86%	64		72%
Total	93		100%	89		100%

Table II. The frequency of papillary thyroid microcarcinomas with potential evolution toward clinically overt thyroid cancer (multifocality ± extrathyroidal extension ± aggresive histologic variant ± regional lymph node metastases)

	Group 1: Papillary thyroid microcarcinoma without autoimmune thyroid disease	Group 2: Papillary thyroid microcarcinoma with autoimmune thyroid disease
Microcarcinomas with potential evolution toward clinically overt thyroid cc.	26 (40.6%)	7 (28%)
Microcarcinomas without this potential transformation	38 (59.4%)	18 (72%)
Total	64 (100%)	25 (100%)

Although the frequency of potential aggressive microcarcinomas without autoimmune thyroid disease (40.6%) was higher than in group with autoimmune thyroid disease (28%), the difference was not significant (p=0.33).

According to the 6th edition of the American Joint Committe on Cancer/International Union against Cancer, TNM staging system varies with cell type, tumor size, extrathyroidal extension, lymph node metastases, distant metastases and age at time of diagnosis.[21,22] In our groups the mean age of patients having only papillary thyroid microcarcinoma (50.5±13.3 years) was significantly higher than that of patients with microcarcinoma associated with autoimmune thyroid disease (43.7±12,0 years) (p=0.05).

Patients older than 45 years with lymph node metastases and/or extrathyroidal extension are included in stage III, and subjects younger than 45 years without distant metastases are in stage I.[23,21] Among patients with papillary thyroid microcarcinomas without any autoimmune thyroid disease 17.2% (11/64) were in stage III, and from thyroid cancer patients with autoimmune thyroid process just one woman was in stage III (1/25). All the other cases were included in stage I.

Microcarcinomas are commonly considered T1 in TNM-system[21,16], but those with extrathyroidal extension (e.g. extension to perithyroid soft tissues) are T3. Microcarcinomas usually are at a very low risk, but those being included in T3 or involving lymph node metastases are included in the high-risk group.[16] The high-risk group have a different therapeutical management versus patients being at a very low-risk.[16,19] Near-total thyroidectomy, completion thyroidectomy should be proposed in the case of a large tumor, multifocality, extrathyroidal extension and/or local or distant metastases, unfavorable histology.[5,10,15] Thyroid carcinomas with extension beyond the thyroid capsule or lymph node involvement have a definite indication for postsurgical administration of ^{131}I.[4,16]

We determined the average diameter of microcarcinomas, and we observed a significant difference between advanced stages (III) and I stage (10.8±3.9 cm vs. 6.9±4.5 mm, p=0.008, 95% CI: 1.03–6.89).

CONCLUSIONS

Autoimmune thyroid diseases were associated significantly more frequently with papillary thyroid microcarcinomas (28%) than with the clinically overt forms (14%). The mean age of patients with papillary thyroid microcarcinoma alone (50.5±13.3 years) was significantly higher than that of the patients diagnosed with autoimmune thyroiditis and microcarcinoma (43.9±10.7 years). The higher rate of occult tumor in patients with papillary thyroid carcinoma and Hashimoto's thyroiditis, and the younger age in cases of microcarcinomas coexisting with autoimmune thyroid disease might suggest that the autoimmune thyroid process is a defence reaction against malignant thyroid lesion. The immune processes are more active in younger people, and this might contribute to the better prognosis of thyroid microcarcinomas (compared to clinical overt cancers), predominating in young patients.

37% of papillary thyroid microcarcinomas were multifocal, had extrathyroidal extension, aggressive histological variant and/or regional lymph node metastases, these microcarcinomas presenting a high risk to transform into clinically overt thyroid carcinomas. Although the frequency of these microtumors without autoimmune thyroid disease (40.6%) was higher than in group with autoimmune thyroid disease (28%), the difference was not significant. In accordance with the 6th edition of TNM-staging system, 17.2% out of patients with papillary thyroid microcarcinomas alone, and 4% out of patients with microcarcinoma and autoimmune thyroid disease were in stage III (high-risk group), which is an important aspect in the later

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Study concerning gastritis in children at the Pediatric Department Number One from the Clinical Hospital of Emergency, Mureş County

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Gastritis is the inflammation of the gastric mucosa supported by histopathologic arguments. Etiologically, the two forms of gastritis are primary gastritis (caused by Helicobacter pylori infection or bile reflux) and secondary gastritis (burns, head trauma, systemic deseases, infections, drugs, caustic and corrosive substances). Gastritis can be acute or chronic. More risk factors are implicated. Helicobacter pylori is considered nowadays the most frequent cause of gastritis.
The diagnosis of gastritis is based on the endoscopic, histological, topographic criteria of the Sydney System. The most symptoms are : abdominal pain, nausea, vomiting.
The study were included 60 children aged 0 -18, from the Pediatric I Clinic Târgu-Mureş between 2007-2008. In our group the frecquency of gastritis was higher in females, and the most affected was the 14 -18 year old age group. At the endoscopic examination acute gastritis was observed in 26 cases (43,33%). Helicobacter pylori was observed in 31 cases (51,66%).
***Key words**: gastritis, child, Helicobacter pylori, endoscopy*

Gastritis is the inflammation of the gastric mucosa supported by histopathologic arguments acquired by endoscopic biopsy [8,9].

From etiologic point of view there are two types of gastritis: the primary one (caused by Helicobacter pylori or bile reflux) and secondary one (caused by infections, drugs, chemicals, caustic/corrosive substances, burns, head trauma and systemic diseases) [8,10].

Gastritis can be either acute and chronic: the acute ones can be diagnosed at any age : acute hemorrhagic gastroduodenitis of the newborn, viral, bacterial, fungal, drug related, allergic (allergies caused by the cow milk proteins), corrosive gastritis. Chronic gastritis can be interstitial and hypertrophic, infiltrative and/or major hypertrophic and atrophic[8].

The etiology is multifactorial, involving several factors like: *mechanical aggression* (tough cellulose abuse, insufficient mastication); *physical aggressions* (the ingestion of too warm or too cold food); *chemical aggressions* (nonsteroid anti-inflammatory drugs, corticosteroids, cytostatics, corrosive substances, smoking, alcohol)[1]; *food-hygiene abuses* (the use of spices, fried fat, carbonated drinks, acid juices, chaotic meals); *psychological factors* (psycho-affective lability, stress); excessive physical efforts[8,10,11]. An important part of these risk factors have a role also in the occurrence of gastric cancer. Some studies proved/showed that Helicobacter Pylori, as well as smoking, low socio-economic level, the infection with Helicobacter pylori, haemorrhagic, hypertrophic with the creases' hyperplasia)[6,8]; histological (acute gastritis- the presence of infiltration with neutrophyl in the mucosa and chronic gastritis with lymphoid infiltration)[8,10,11]; topographic (antral gastritis, the gastritis of the gastric body, pangastritis with or without antral or corporal predominance)[8].

MATERIAL AND METHODS

We performed a descriptive study focused on the investigation of some variables concerning the person (gender, age, place of living), clinical symptomatology, the etiology of gastritis, study that included 60 children from the Pediatric I Clinic, Târgu-Mureş in the period October 2007 - December 2008. First of all, the purpose of this study is to establish the dimension of the phenomenon (the frequency by the case definition), the severity of the histological confirmed gastritis, the severity of some risk factors especially of the ones with Helicobacter pylori and feeding.

The including criteria were: a) patients with age between 0-18; b) clinical symptoms (epigastric pain, nauseas, anorexia); c) the outcome of endoscopy that confirms the gastritis.

Research methods: a) the elaboration of the questionnaire in order to collect the data; b) to fill in the

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questionnaire by direct interview with the patients or their family members; c) endoscopic biopsy – the common way of biopsy sampling from the gastro-intestinal tract' lesions; d) the microscopic examination of the samples from different tissues and e) the final report made by the anatomopathologist with all the pathological elements that can be viewed.

We analyzed and registered demographic, anamnesis and clinical data as well as the endoscopic bulletins and the histopathological outcomes of the patients from this study.

RESULTS

The distribution of the cases concerning their place of living shows predominance especially in the children from rural places (55%).

We observed a higher frequency of gastritis in females - 40 cases (66,6%) when compared to males - 20 cases (33,34%). (Figure 1)

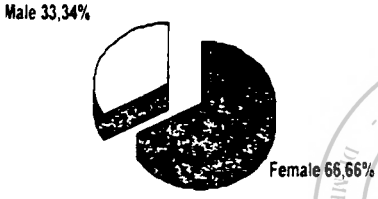

Figure 1. The distribution of cases based on gender

The distribution of cases based on age groups, showed a higher frequency of gastritis in children with age above 14 years (46,66%) and the age group 10 -14 (36,66%) with a net predominance in females. (Figure 2).

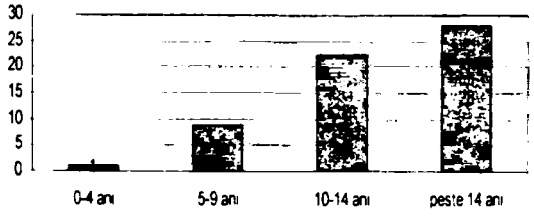

Figure 2. The distribution of cases based on age groups

The infection with Helicobacter pylori was observed in 36 cases, the highest percentage (44,44%) being registered in the group with ages between 14 -18 (16 cases). (Table I)

Table I. Helicobacter pylori infection

Age	Cases
0-14 ys	1
5-9 ys	5
10-14 ys	14
14-18 ys	16

Concerning the associated risk factors it we noticed the presence of gastric pathology (gastritis, ulcer) in the family members in 19 cases (31,66%).

Inadequate feeding (spices, fried fats, carbonated juices, chaotic meals, inefficient mastication), emotional stress, fear of failure are present in many cases. (Figure 3)

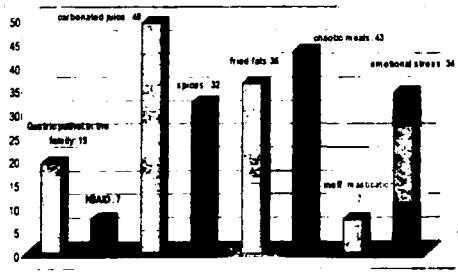

Figure 3 . Risk factors of gastritis

We also noticed a seasonal evolution of the gastritis with two high points: in springtime with a percentage of 31,66% (19 cases) and in autumn with a percentage of 56,66% (34 cases).

In the studied lot we noticed a higher frequency of abdominal pain (83,33%), inappetence, nausea and vomiting. Pain, most frequently was epigastric (64% cases). 16,66% of the patients didn't have abdominal pain at all. (Table II)

Table II. Symptoms of gastritis

Symtoms	Cases
Abdominal pain	50 (83,33%)
Inappetence	28 (46,66%)
Nausea + vomiting	24 (40,00%)
Painful hunger	23 (38,33%)
Loss of weight	16 (26,66%)
Nausea	14 (23,33%)
Vomiting	8 (13,33%)
Hematemesis	3 (5,00%)

Endoscopical examination, antral gastritis was predominant (29 cases), gastritis of the gastric body (14 cases) and pangastritis has been observed in 16 cases. (Figure 4)

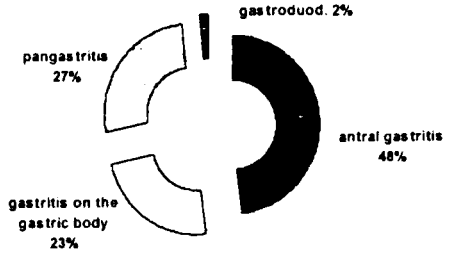

Figure 4. Topographic gastritis

The histopathologic examination showed the presence of infiltration with polymorphonuclear substances (acute gastritis) in 26 cases (43,33% cases) and the presence of infiltration with lymphomonocytes (chronic gastritis) in 34 cases (56,66%).

DISCUSSIONS

The frequency of gastritis can nowadays be way appreciated correctly due to the modern techniques of investigation; using gastroscopy one can view also the superficial injuries of the mucosa, which otherwise couldn't be noticed at the radiological examination. In the same time, endoscopy allows an early diagnose of the cases. [10]

We noticed a two times higher frequency of gastritis in females, similar data being mentioned also by other authors. [8]

Making the connection with the age, the most affected part of the population was the age group of 14 - 18, almost half from the patients of this study. In another study (Goldman et al) mean age was 10+/-3. [4]

Helicobacter Pylori infection is very important, this being proved by the presence of this bacteria in the bioptic material of over 50% of the cases.

Golden et al , in a study with 395 children with gastrointestinal symptoms noticed a higher incidence of Helicobacter pylori (40%) , children from families with antecedents of gastritis had a higher risk of being colonized with H pylori ($p<0,01$). [4]

Cambell et al showed the importance of this infection in the etiology of children gastritis [2], and Egan et al noticed the most frequent of antral gastritis. [3]

In the study of Khean-Lee Goh et al, the following factors were found to be significant for gastric cancer: Helicobacter pylori infection, low level of education, smoking, incorrect feeding [7]

Other reasons that facilitate the appearance of gastritis are: incorrect feeding and living style but stress is also frequently invoked. [9]

Therefore, gastritis in teenagers and young adults can be considered a multifactorial disease, the bacterial component interfering with some other factors which can increase the risk of the disease's appearance. Some of these risk factors, like feeding and stress are modifiable. [11]

CONCLUSIONS

1. Gastritis is more frequent in female.
2. The most affected group of age is 14 -18
3. The infection with Helicobacter pylori was the most frequent cause of gastritis.
4. An elder child has in most of the cases typical clinical picture, while the younger child has an unspecific symptomatology.
5. There was remarked a seasonal evolution of the disease with two morbidity peaks: spring time and autumn.

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Linking the endothelial dysfunction in patients with metabolic syndrome with and without diabetes mellitus type 2

Andreea Iana[1], R. Timar[2], C. Tudor[3]

The metabolic syndrome is a highly prevalent multifaceted clinical entity produced through the interaction of genetic, hormonal, and lifestyle factors. Type 2 diabetes mellitus is increasingly prevalent worldwide, conferring major burdens on health and health care costs. Development of atherosclerotic cardiovascular disease (CVD) is the principal complication in type 2 diabetes mellitus and a major cause of morbidity and mortality. Endothelial dysfunction is a systemic disorder and a key in the pathogenesis of atherosclerosis and its complications.
The aims of our study were to evaluate the influence of diabetes mellitus type 2 on the endothelial dysfunction in the metabolic syndrome without clinical changes of CVD.
***Key words**: diabetes, metabolic syndrome, endothelial dysfunction, atherosclerosis*

The increasing prevalence of obesity has led to a comparable increase of type 2 diabetes, a major cause of death, morbidity and disability.

Metabolic syndrome causes moderate increases in all-cause and CVD mortality.[8] The normal endothelium regulates the vascular homeostasis through the release of vasodilatation factors, such as nitric oxide, prostaciclin and others vasoconstrictor one such as endothelin, superoxid anions or angiotensin II [3]. Endothelial disorder is a generally term which implies the decrease of nitric oxide synthesis and / or an imbalance between relaxing factors and vasoconstriction [4]. It is also known that endothelial tissue is an active and dynamic tissue, essential to maintain the cellular homeostasis, thrombosis and fibrinolizis - mechanisms that are affected by a significant imbalance in diabetes (platelets expose more IIb / IIIa receptors and have a greater tendency to aggregation, especially by hyperglycemia, and in terms of coagulation mechanisms is well known that diabetic patients present an increased concentration of fibrinogen and a reduction of the activity of the tPA, increasing concentration of secondary PAI1 – an inhibition factor of the activation factor of plasminogen).[1] Endothelial dysfunction reflects a vascular phenotype prone to atherogenesis and may therefore serve as a marker of the inherent atherosclerotic risk in an individual. It is a systemic disorder and a critical element in the pathogenesis of atherosclerotic disease and its complications. Arterial stiffness is increased in patients with metabolic syndrome irrespective of the definition criteria.[11]

MATERIALS AND METHODS

Studied population:

The study enrolled 84 patients with metabolic syndrome, 53 men (63,1%) and 31 women (36,9%) with the mean age of 50.81±7,91years. The metabolic syndrome was defined according to the IDF criteria and diabetes mellitus type 2 according to the WHO criteria.

Exclusion criteria's:

- smokers
- chronicle arteriophaty of lower limbs
- cerebrovascular disease (stroke)
- coronary disease
- diabetes treated by insulin
- kidney and liver failure
- psychiatric disorders
- malignancies
- consumption of alcohol per day over 30gr

Methods: as method to assess the endothelial dysfunction was used the flow mediated vasodilation.

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Table I
Characteristics of the study group

Parameters	MS without DM type 2	MS with DM type 2
Abdominal wa.st	114,21± 12,60	113,59 ± 12,26
BMI	33,4 ± 38,46	32,4 ± 5,46
HDL	42,52 ± 12,79	37,07 ± 9,15
Tryglicends	165,74 ± 70,17	250,76 ± 187,67
Glycemia	105,02 ± 8,60	170,38 ± 55,92

Table II

t-Test: Two-Sample Assuming Unequal Variances for FMD%

	3,9216	-1,9608
Mean	7,2263	3,9251
Variance	64,2598	28,3807
Observations	53,0000	28,0000
Hypothesized Mean Difference	0,0000	
df	75,0000	
t Stat	2,2126	
P(T<=t) one-tail	0,0150	
t Cntical one-tail	1,6654	
P(T<=t) two-tail	0,0300	
t Critical two-tail	1,9921	

The ultrasound was equipped with vascular software for recording 2-D color Doppler and a spectral and linear vascular probe with adjustable frequency between 7 and 12 MHz. The vascular diameter in systolic and diastolic longitudinal plane was measured guided by the principle of "leading-edge", followed by determination of basal medium velocity by pulse Doppler (average of at least 3 determinations). To obtain stimulate blood flow in the brachial artery is placed the sphygmomanometer antecubital at the forearm. After recording the basal velocity average, the sphygmomanometer cuff is swollen above the systolic blood pressure (usually over 50 mmHg) for 5 minutes, to achieve brachial artery ischemia and dilation of the resistance vessels downstream through the mechanism of self. Sudden deflation of the cuff increases the blood flow in brachial artery (active hyperemia) and the shearing stress at this level with consecutive dilation of the brachial artery. The maximum velocity is measured by pulse Doppler in first 15 seconds of cuff deflation and maximum diameter of the brachial artery is determined at 45-60 seconds post hyperemia.[7],[10] The brachial artery diameter was measured in the same cardiac cycle to avoid variations caused by arterial compliance.

We measured the flow-mediated dilatation (FMD) of the brachial artery (endothelium dependent vasodilatation) on B-mode ultrasound images, in terms of fasting for 8 hours.

Endothelial dysfunction was diagnosed if flow-diated dilatation was less than 10%.

RESULTS AND DISSCUSIONS

Diabetes mellitus type 2 was present by 29 patients (34, 5%) with metabolic syndrome, they had a corporeal mass index of 32,4 ± 5,46kg/m2 with an average abdominal circumference of 113,59 ± 12,26cm.

In literature increasing body weight favors the clustering of coronary heart disease risk factors. Overweight and obesity, however, do not independently associate with carotid atherosclerosis. [6]

The mean value of FMD% by patients with metabolic syndrome without diabetes mellitus type 2 was 7,22% and by patients with metabolic syndrome and diabetes mellitus type 2 was 3,92% with a P(T<=t) one-tail = 0,0149.

To obtain the results was performed the test t-odd: (Table II)

The P = 0.0149 value observed in Table I is significant from the statistical point of view; there is an influence of diabetes type 2 on endothelial function in metabolic syndrome by the studied group. As we know the patterns of metabolic syndrome components and the longitudinal changes that lead to the metabolic syndrome are different in men and women. Interestingly, components with the highest prevalence prior to metabolic syndrome development, such as elevated blood pressure, are not necessarily the stronger risk factors[9] such as plasma glucose levels. But intensive glucose control in patients with poorly controlled type 2 diabetes had no significant effect on the rates of major cardiovascular events, death, or microvascular complications.[2] However currently

available evidence suggests that therapeutic efforts that target hyperglycemia early in the course of diabetes have a long-term cardiovascular benefit. [5]

CONCLUSIONS

Diabetes mellitus type 2 by patients with metabolic syndrome increased the endothelial dysfunction. Endothelial dysfunction may be an attractive primary target in the effort to optimize individualized therapeutic strategies to reduce cardiovascular morbidity and mortality.

The detection of the early endothelial dysfunction and population at high risk may lead to proper treatment with improvement of the life quality.

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Painkillers or sport to alleviate chronic pain in endometriosis?

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Study objective was to assess potential individual factors influencing quality of life and pain scores of patients suffering from histologically confirmed endometriosis in a university-based Gynecology department. We used a questionnaire among patients of reproductive age undergoing laparoscopy with a presumed diagnosis of endometriosis. Details of fertility, previous treatments and quality of life, sexual activity, as well as linear pain scores using a visual analogue scale for several symptoms, were recorded. Moreover, data were gathered in the following groups of variables: age, marital status, education, reproductive and medical history including previous pregnancies and parity, knowledge of accompanying pelvic disorders, regular sport activity, as well as general quality of life estimates including self-image. Intraoperative findings were also collected and only those data were used where endometriosis was intraoperatively and histologically proven. Data were statistically evaluated. Eighty-one patients complaining about persistent pelvic pain were later intraoperatively and histologically proven to have endometriosis. Thirty-one of them (38,2%) reported regular sport as part of their daily life schedule while 50 of them (61,8%) performed no physical activity at all. Fourteen patients among regular exercisers and 33 patients among those without physical activity reported the effectiveness of painkillers for pelvic pain, corresponding to 45,1% and 66% of these subgroups, respectively (difference statistically significant, p<0.05). Based on our results, we can conclude, that taking painkillers might be less effective among endometriosis patients performing regular daily sport activities, and, thus it might impose them to an unnecessary burden of possible side effects.
Keywords: endometriosis, pelvic pain, exercise, painkillers

Endometriosis affects millions of women world wide. It can severely alter quality of life and leads to extensive problems with fertility and loss of work time.[4] endometriosis might remain asymptomatic and discovered accidentally. However, it may cause symptoms, which include chronic pelvic pain, bleeding, infertility, and increases susceptibility to development of adenocarcinoma.[6] Signs and symptoms arise from cyclic bleeding into the surrounding tissues, resulting in inflammation and formation of scarring and adhesions. It is peculiar, that symptom severity does not correlate well with the extent or progression of the lesions.[1] Minor laparoscopic findings might be associated with severe complaints, while large lesions might remain undetected and revealed only accidentally. The exact roles of different factors contributing to the establishment and persistence of the endometriotic lesion are not yet fully understood. Despite the high health care costs and the associated morbidity, the incidence, prevalence, and risk factors of endometriosis, as well as factors influencing the effectiveness of therapy, remain uncertain.

Symptomatic endometriosis can be managed surgically and/or medically. The aim is pain relief and/or amelioration of infertility. Medical treatment is usually long term, and recurrence is frequent after its cessation. Classic endometriosis pharmacotherapy is represented by GnRH agonists, oral contraceptives and Type II progesterone receptor ligands.[13] All medical treatments seem to be equally effective in managing endometriosis. Although about 80-85% of patients have improvement in their symptoms[5], many women experience unsatisfactory results. However, little is known about factors on patient's side influencing the efficacy of generally accepted therapeutic approaches used to alleviate symptoms caused by endometriosis.

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In our study, we investigated possible individual and environmental factors that might alter the effectiveness of therapy for pain relief among patients with histologically confirmed endometriosis. To determine these parameters we used a questionnaire before laparoscopic surgery for persistent pelvic pain and later we included data from those patients who were diagnosed intraoperatively and histologically with endometriosis. With that, we tried to determine factors influencing pain and pain relief, with focus on several non-medical variables such as marital status, level of education and regular sport activities.

MATERIALS AND METHODS

Study population and sample. Study population primarily consisted of a cohort of 150 patients of reproductive age presenting persistent pelvic pain and undergoing laparoscopy to explore the underlying reason. Prior to the operation all patients consented to participate in the study. A standardized questionnaire elicited information from women on the following groups of variables: age, marital status, education, reproductive and medical history. The questionnaire was purposefully designed to ascertain information on potential confounders, which included gravidity (number of pregnancies regardless of outcome) and parity (number of live births), knowledge of accompanying pelvic disorders, and caffeine intake, since all have been reported as risk factors for endometriosis.[1,9,2] Further variables were concurrently used medication including pain killers, as well as daily habits of exercise and sport, type of work and general quality of life estimates including self-image. Pelvic pain was scored using a visual analogue scale from 0-10. Only data from patients with histologically confirmed endometriosis and with no other pelvic/abdominal alteration or disease confirmed at laparoscopy were later processed in the study.

Operative procedures. Laparoscopies were performed by highly trained and experienced surgeons. Following the operations they completed a standardized operative report to ascertain information on postoperative diagnosis and other pathology regardless of surgical indication. Severity of endometriosis was staged according to the American Fertility Society's revised definition. In all patients endometriosis lesions were laparoscopically removed and/or electrocauterized and histological examination confirmed diagnosis. The affiliated University gave Institutional Review Board approval for the conduct of this study.

Statistical analysis. Analysis of data was performed using Microsoft Excel and SPSS 15 programs. We applied chi-square test, analysis of variance (ANOVA), and Pearson-Spearman's rank correlation test. Data are presented as percentage values.

RESULTS

The number of eligible patients with intraoperative and histological diagnosis of endometriosis was 81. Mean age of them was 31,2 years. Among them, 31 patients (38,2%) reported regular sport as part of their life, while 50 patients did not report any significant physical activity (61,8%). Comparing subgroups of those regularly performing sport with patients without physical activity, the effectiveness of painkillers for pelvic pain was reported by 14 patients (45,1%) and 33 patients (66%), respectively ($p<0.05$, Figure 1.) (page 9). In addition to that, average pain scores were not different in these subgroups. Detailed analyses revealed no significant differences in pain and quality of life measures with relation to marital status, level of education, number of previous pregnancies regardless of outcome and number of live births. Moreover, analyzing the data concerning the extent of the disease (i.e. the stage of endometriosis recorded at laparoscopy) as well as quality of life values revealed no significant correlations.

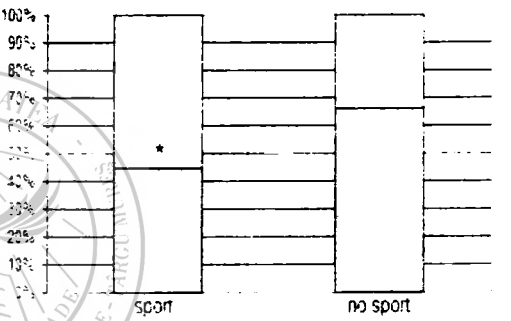

Figure 1. Correlation between regular exerciser status and effectiveness of painkillers among endometriosis patients with chronic pelvic pain. Data are presented as percentage values. Gray bars represent the percentage of patients within the corresponding subgroup responding positively to painkiller therapy; sport, subgroup of patients performing sport activities on a regular basis; no sport, subgroup of patients with no regular exercise; *$p<0.05$

DISCUSSIONS

The currently available medical treatments for endometriosis seem to be equally effective. It is estimated that about 80-85% of patients have improvement in their symptoms.[11] Interestingly, severity of symptoms does not correlate well with the extent of the disease[1], although, in a well conducted study of 63 women a benefit from conservative surgery seemed to be greater in those with the most severe disease.[12] Unfortunately, there were only two participants in the group with severe disease, so limited data might hinder us to draw firm conclusion. In general, we know little about possible environmental and individual factors that can negatively influence the efficacy of different therapeutic modalities.

In our study we observed an inverse relation between regular sport activity and effectiveness of painkillers for pelvic pain in endometriosis patients. Although the observed difference was not striking

(approximately 21%), it was found to be statistically significant. For the first sight this result might be surprising. It is known that exercise of sufficient intensity and duration increases circulating beta-endorphin levels.[7] Beta-endorphin is an important reliever of pain.[3] In a study by Vercellini and co-workers, patients undergoing laparoscopy for chronic pelvic pain and/or infertility were studied to elucidate whether beta-endorphin concentrations in peripheral mononuclear cells differed according to the presence or absence of endometriosis.[15]

This paper demonstrated significantly lower beta-endorphin levels in the endometriosis group than in the controls.

Moreover, it appears that the psychological profile of symptomatic endometriosis patients does not differ from those chronic pelvic pain patients who have a normal pelvis or other gynecological conditions. Thus, it seems, that local biochemical and physical effects of endometriotic lesions are the most important factors in determining frequency and severity of symptoms.[14]

Furthermore, there are numerous data demonstrating that serum level of leptin, a protein with a structure similar to cytokines that assists in the regulation of body weight and energy homeostasis, is significantly reduced during long-term (more than 12 weeks) training in females.[8] In addition, the study of Matarese and co-workers demonstrated a statistically significant increase in leptin levels in serum and peritoneal fluid of patients with endometriosis, compared with control population.[9] Summarizing above data, it can be easily hypothesized, that, regular sport activities can affect pain scores and overall well being of endometriosis patients through different mechanisms of action, i.e. increasing circulating beta-endorphin levels and lowering serum and peritoneal fluid leptin concentrations.

Thus, we might explain why painkillers were reported less likely to be effective among those performing regular exercise. Since mean pain scores were not different among the subgroups, the decreased efficacy of painkillers among patients with regular sport activities might result from the absence of additive effect of the drugs to physical exercise instead of the absence of their effect per se.

Moreover, in a study by Dhillon et al., women who reported frequent, high-intensity activity for a 2 years period had a 76% reduced endometrioma risk as compared with women who engaged in no high-intensity activity.[5]

CONCLUSIONS

Based on above, we can conclude, that, beyond classic endometriosis pharmacotherapy and surgery, regular exercise or sport might have an advantageous impact on the overall well being of patients with chronic pelvic pain caused by endometriosis. Although our data passed strict statistical analyses, because of their limited amount, we consider this work as a preliminary one to initiate specially aimed international studies to further clarify the issue.

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New aspects in adenocarcinoma of the esophagus and Barrett's esophagus

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Purpose: Esophageal cancer is encountered more frequent than previously reported. Adenocarcinoma and squamos cell carcinoma account for the majority of esophageal neoplasms. We studied the incidence of esophageal cancer and Barrett's esophagus in our geographic area.

Matherial and method: We studied a series of 2633 patients who were investigated by upper endoscopy in the Gastroenterology Departement.

Results: In 61 patients a diagnosis of cancer was established (46 gastric and 15 esophageal). There was a strong predominance of males for esophageal cancer. The mean age at diagnosis was 56 years. Most tumors were located in the distal esophagus. Squamous carcinoma comprised 66,66% of all cases. It can be assumed that, at least compared to gastric cancers patients in our series, the incidence is higher than a few decades ago. Our results show that the mean age at diagnosis is lower than in western countries and squamous cell carcinoma is still the most prevalent.

Conclusions: Esophageal cancer is increasing in our country too, his epidemiology reflecting the risk factors in general population.

Key words: *adenocarcinoma, squamos carcinoma, Barrett's esophagus*

Esophageal cancer, one of the deadliest cancers worldwide, is encountered more frequent than previously reported. Two histologic types account for the majority of malignant esophageal neoplasms: adenocarcinoma and squamous cell carcinoma. In the 1960s, squamous cell cancers comprised more than 90% of all esophageal tumors. The incidence of esophageal adenocarcinomas has risen considerably over the past 2 decades, such that it is now more prevalent than squamous cell cancer in the United States and Western Europe, with most tumors located in the distal esophagus.[1,9] From 1975 to 2001, the incidence of esophageal adenocarcinoma rose approximately sixfold in the United States (from 4 to 23 cases per million), a relative increase greater than that for melanoma, breast, or prostate cancer. [3] While risk factors for squamous cell carcinoma of the esophagus have been identified such as tobacco use, alcoholism, malnutrition, infection with human papillomavirus[3] the risk factors associated with esophageal adenocarcinoma are less well defined. The most important epidemiological difference between squamous cell cancer and adenocarcinoma, however, is the strong association between gastroesophageal reflux disease (GERD) and adenocarcinoma. The results of a population-based case controlled study suggest that symptomatic gastroesophageal reflux is a risk factor for esophageal adenocarcinoma. The frequency, severity, and duration of reflux symptoms were positively associated with increased risk of esophageal adenocarcinoma.[5] GERD is a risk factor for esophageal adenocarcinoma because long-standing GERD is associated with Barrett's esophagus, the condition in which an abnormal intestinal epithelium replaces the stratified squamous epithelium that normally lines the distal esophagus.[6]

We studied the incidence of esophageal cancer and Barrett's esophagus in our geographic area.

MATERIAL AND METHOD

We studied a series of 2633 patients who were investigated by upper endoscopy in the Gastroenterology Departament between 1 January 2008 and 31 December 2008. In all cases with abnormal aspects at endoscopy multiple biopsies were made and a pathologic diagnosis was available. Were included all newly diagnosticated patients, with a definitive pathologic diagnosis. The patients with previous gastrectomy, patients with previous diagnosis of esophageal cancer and patients with unclear pathologic diagnosis were excluded. The results were compared with a series of 2715 patients examined in a similar period of time, between 1 January 2006 and 31 December 2006.

The data were collected with Microsoft Excell programe and analised with GraphPad Instat programe.

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RESULTS AND DISCUSSIONS

In 2008 series of patients in 61 (15 women and 46 men) a diagnosis of cancer was established (46 gastric cancers and 15 esophageal). There was a strong predominance of males (13 cases) for esophageal cancer (Figure 1)

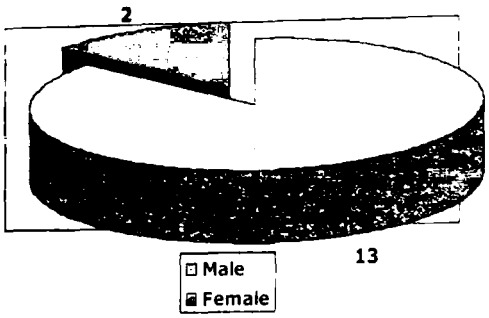

Figure 1. Distribution of esphageal cancers by gender

The mean age at diagnosis in these 15 cases was 56 years. The younger patient with esophageal cancer was only 35 years old (Table I).

Table I.Distribution of esophageal cancers according to age groups

Age group	Nmber of patients
under 40	1
40-50	1
50-60	9
60-70	2
70-80	2

Barrett's esophagus was diagnosed in 16 patients, with moderate displasia in 3 patients. The incidence of Barrett's esophagus was also higher in men, with 12 cases compared to 4 in women (Figure 2).

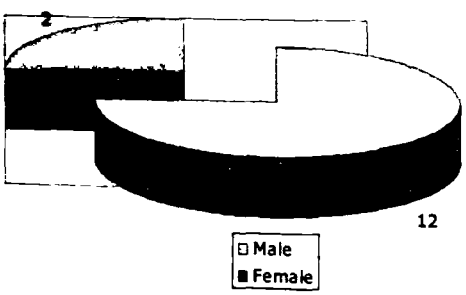

Figure 2. Distribution by gender of patients with Barrett's esophagus

Most tumors (11 patients - 73,3%) were located in the distal esophagus. Squamous carcinoma comprised 66,6% of all cases. All adenocarcinomas (5 patients) were located in the distal esophagus (Table II).

Table II. Localisation of esophageal neoplasms

Localisation	Number of patiens
Distal esophagus	11
middle	2
proximal	2

Compared to these results, in 2006 were performed 2715 upper endoscopy examinations, with 42 gastric cancers, 13 esophageal cancers (11men and 2 women) and 10 patients with Barrett's esophagus. Two of them were with severe displasia.

The incidence of Barrett's esophagus in both sexes (16 cases) was increased compared to refference period (only 10 cases).

Although this study can not calculate the real incidence of esophageal cancer in general population, it can be assumed that, at least compared to gastric cancers patients in our series, the incidence is higher than a few decades ago.

Our results show that the mean age at diagnosis is lower than in western countries, 56 years compared to 60 in the study of Tomizawa.[9] In our study squamous cell carcinoma is still the most prevalent. As oposed, in western countries esophageal adenocarcinoma is now the most prevalent histologic type.[9]

The high men to women ratio in our study correlates with increased incidence of squamos cell carcinoma in our area, compared to 3,5/1 men-women ratio for adenocarcinoma in western countries.[2]

In our study three patients with Barrett's esophagus had moderate dysplasia and in one patient adenocarcinoma developed after Barrett's esophagus. Surveillance of affected patients with Barrett's esophagus for the development of dysplasia and adenocarcinoma, potentially allows for early diagnosis of esophageal carcinoma or even prevention of cancer by ablation of dysplastic lesions.[7]

CONCLUSIONS

Upper digestive endoscopy must be performed at all males patients, regardless of age, with disphagia, weight loss, esophageal reflux disease or with personal preneoplasic lesions.

Adenocarcinoma af the esophagus and Barrett's esophagus is increasing in our country too.

The squamous cell carcinoma is still the most prevalent, in accordance with high incidence of smoking, alcohol intake and low income.

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Diagnosis of *Clostridium difficile* associated disease

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Purpose: Evaluation of Clostridium difficile (C. difficile) involvement in the aetiology of hospital acquired diarrhoea.
Material and method: During 01.11.2008 – 29.02.2009, in the Microbiology Laboratory from Mureş Emergency County Hospital, we analysed 170 samples of stools according to the laboratory's coproculture protocol (this didn't include testing for C. difficile). From these, we selected 22 diarrhoeic samples suggestive for C. difficile infection, which were analysed for the presence of C. difficile toxins A, B. The positive samples were cultivated on C. difficile agar in anaerobic atmosphere. The identification was based on the microscopic morphological characters, the colonies' aspect and the latex agglutination test.
Results: From the 170 samples processed by coproculture we identified 3 positive samples (1.76%). From the 22 stools tested for C. difficile, 5 were positive (22.7%).
Conclusions: The percentage of the positive samples for C. difficile is significant higher than the one obtained for community enteric pathogens. The detection of C. difficile sustains the importance of routine testing for this species. The rigorous selection of the stool samples has an important role in balancing the costs and the efficiency for this diagnosis
Key words: *Clostridium difficile, coproculture, diarrhoea*

Clostridium difficile (C. difficile) associated disease (CDAD) has been described in the literature since the early 1980s. Although important work has been accomplished on the epidemiology, clinical diagnosis and control of hospital outbreaks, CDAD continues to persist as a costly leading cause of nosocomial gastrointestinal illness.[4]

C. difficile is now thought to be responsible for a wide range of diseases from asymptomatic colonization, to diarrhoea of varying severity, life-threatening colitis, often as a consequence of antibiotic exposure. Effective control of CDAD in the hospital requires both antibiotic control and prevention of environmental seeding and bacterial spread.[8]

C. difficile is a Gram positive, spore forming anaerobic bacillus. It is widely distributed in the environment and colonizes up to 3 – 5% of adult humans without causing symptoms. Certain strains can produce two toxins: enterotoxin A, which is mainly responsive for diarrhoea, and cytotoxin B.[5] A recently identified strain of C *difficile* (NAP 1) that has caused numerous outbreaks of clinically severe disease in North America and Europe produces 16 times more toxin A and 23 times more toxin B than other strains. In addition, NAP 1 produces a third toxin, known as binary toxin, although its significance is unknown.

C. difficile toxins can be found in the stool of 15% to 25% of patients with antibiotic associated diarrhoea and more than 95% of patients with pseudomembranous colitis.

Data from the US Centers for Disease Control and Prevention (CDC) revealed that hospitalizations with a discharge diagnosis of CDAD have significantly increased from 31 per 100,000 population in 1996 to 61 per 100,000 in 2003.[10] These changes in the incidence and severity of CDAD may be associated with the emergence of a more virulent strain of *C. difficile*. Death rates associated with *C. difficile* were reported to be increasing from 1999 to 2002 in the United States and from 2001 to 2005 in England and Wales.[7] Mortality of CDAD ranges from 6% to 30% when pseudomembranous colitis is shown to be present, and is substantial even in the absence of colitis.[5] Moreover, the severity of observed disease may also be increasing, with an attributable 1-year mortality rate approaching 17% in one study.[10]

Due to the lack of a national diagnostic protocol for CDAD, at the moment the incidence of this infection in our country cannot be compared to the multitude of existing data at European level and worldwide.

Unfounded use of antibiotics has led to a significant increase in microorganism's drug resistance. This led to the necessity of using some broad spectrum antimicrobial agents more often, thus maintaining a vicious circle. A major side effect of the unfounded antibiotherapy is a significant increase in the incidence of antibiotic induced diarrhoea, but its aetiology is not sufficiently studied in our country yet.

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All these data support once again the necessity for routine testing for CDAD, which would bring a real benefit to the quality of medical act in medical facilities.

Purpose. Assessing the involvement of *C. difficile* infection in the aetiology of hospital acquired diarrhoea.

MATERIAL AND METHOD

The study was performed during 01.11.2008 - 28.02.2009.

During this period, in the Laboratory of Microbiology from Mureș Emergency County Hospital the stool samples were processed following the coproculture laboratory protocol (which includes testing for pathogenic strains of *Escherichia coli, Salmonella spp., Shigella spp., Campylobacter coli / jejuni, Yersinia enterocolitica*). Some of these samples were selected for *C. difficile* testing, according to the following criteria:

-diarrhoeic stool

•from patients hospitalized for 3 days or more

•from newly admitted patients with risk factors and suggestive symptoms

-samples from patients aged under 2 years were not processed.

These criteria were set according to data from specialty literature, in order to avoid unjustified tests.

The samples selected for the diagnosis of CDAD were processed according to the following protocol:

-The stools were tested for the presence of toxins A and B produced by *C. Difficile*

•the test was performed using MiniVidas system (Biomerieux) from Microbiology Department within the University of Medicine and Pharmacy Târgu-Mureș

-the MiniVidas testing principle combines a method of immunological sandwich-type enzyme in two phases analysis with final fluorescence detection (Enzyme-Linked Fluorescent assay - ELFAA)

-positive samples were then cultured in anaerobic atmosphere on selective media for *C.difficile*

•the culture was obtained on Clostridium difficile agar plates (cycloserine-cefoxitin-fructose agar) (Biomerieux)

•the anaerobic atmosphere was generated using Genbag Anaerob (Biomerieux)

-after obtaining isolated colonies, we identified them, based on:

•microscopic morphological features and Gram staining

•Gram-positive bacilli, with ovalar subterminal spore

•colonies' aspect and smell

-characteristic colonies of approximately 4 mm diameter, round, with edges slightly filamentous

-strong smell of horse manure

•latexagglutination test performed using *C. difficile* Test Kit (Oxoid).

-Identified strains were frozen at – 70 °C.

RESULTS AND DISCUSSIONS

During the study, in the Microbiology Laboratory from Mureș Emergency County Hospital were processed 170 stool samples following the laboratory coproculture protocol; for these, 3 samples were positive for enteric community pathogens (a pathogenic strain of *Escherichia coli*, one *Salmonella spp.* and one of *Shigella boydii*), representing 1.7%.

Of the 170 samples, according to the criteria above, we selected for the diagnosis of CDAD 22 samples; we obtained positive results in 5 cases, representing 22.7% (Table I).

Table I

Tested samples		Positive samples	Negative samples
Coproculture	170	3 (1,7%)	167 (98,2 %)
CDAD	22	5 (22,7%)	17 (77,2%)

The low percentage of positive coprocultures for community enteric pathogens can be explained by the hospital's profile, which does not include the Department of Infectious Diseases; this low percentage emphasizes the importance of the CDAD diagnosis, the 22.7% percentage for this positive samples being significantly higher ($p < 0001$).

The rigorous selection of the stool samples processed for *C. difficile* has an important role in obtaining an increased percentage of positive samples for CDAD.

Data from specialty literature suggests that it is important to test diarrheic stools from hospitalized patients for over 3 days and from newly admitted patients with risk factors and suggestive symptoms for CDAD. Some authors even recommend that stools from patients who develop diarrhoea after more than 3 days of admission should be tested for *C. difficile* only.[11] Most laboratories reject diarrheic stools from classical culturing if the sample came from a patient hospitalized for more than 3 days, because the positive rate for enteric community pathogens is extremely low, leading to a low cost-efficiency report. Instead, it forwards the samples to CDAD diagnosis.[2]

Also, testing is not recommended for stools collected from patients under the age of 2 since the colonization of *C. difficile* intestinal tract of the newly-born is more frequent than in adults.[10] A study accomplished in France in 2001 cites a rate of newborns colonization with *C. difficile* between 5 - 70%. Paradoxically, in infants which carriers toxin producing strains of *C. difficile*, symptomatic disease develops rarer than in adults. The reason, based on experiments made on mice, would be the lack of the receptors for toxin A on their immature enterocytes.[1]

CONCLUSIONS

Although the diagnosis of CDAD is considered to be an expensive one, the rigorous selection of the samples that should be tested has an important role in balancing the cost efficiency ratio.

The identification of *C. difficile* in 22.7% of selected samples sustains its involvement in the aetiology of hospital acquired diarrhoea, and the need for routine practice for this diagnosis.

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Down syndrome: informing the parents. A national survey of parental support in Hungarian hospitals

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Objective: Since the 60's several publications dealt with the phenomenon how doctors inform parents of newborns about postnatal recognition of Down's syndrome and the support they receive right after breaking the bad news. However, the rest of these studies concentrated on surveying parental satisfaction, while relatively few international studies deal with the other side of the communicational situation, the opinion of the informer.

Aim: To get an insight to the circumstances of parental information in Hungarian departments and institutions of obstetrics with the help of a questionnaire in order to evaluate the possibilities for interventions.

Methods: The Down's team operating at the University of Pécs Faculty of Health Sciences carried out a national survey in 2005-2006 – an interview-based questionnaire filled by doctors of institutions of obstetrics – with the help of the National Register for Congenital Diseases of the National Centre for Epidemiology and Down's Foundation.

Results: The coverage of the survey reached 74%. Rest of the surveyed institutions did not have information protocol, however, 70% of them believes it would be necessary. Only 44% of the doctors received communication training and 81% of them believe they can manage communication, 33% have already felt that the mother of a newborn with Down's syndrome would expect special help that the institutions are unable to provide. In general the circumstances of the informing were not according to international recommendations.

Conclusion: There are serious problems with the circumstances of parental informing in Hungarian institutions of obstetrics. This situation would obviously require intervention. An aimed communicational training based on international experience and exploiting the openness of doctors, as well as the establishment of information protocol could be elements of such intervention.

Key words: parental informing , Down's syndrome , communicational training

Bad news is all the news that dramatically and negatively changes the patient's vision of future (Buckman 1992).[1] Breaking bad news, is an important and difficult skill. Informing parents about postnatal recognition of genetic defects is a difficult task for medical specialist (Dent and Carey, 2006).[4] There are several ways of breaking bad news and the quality of the informing in a given case may influence the child's entire life, therefore the quality of informing is of key importance. It may influence the parents' feelings for the child and the early bandage (Brinkworth 1975[2], Pugh és Russel 1977[11], Svarstad és Lipton 1977[13], Springer és Steele 1980[14]), it may also make parents mistrustful towards doctors for a long time (Quine and Pahl 1986)[12], and that mistrust may mark the effectiveness of the child's long-term medical treatment (Ley et al.1976).[8]

Down's syndrome is one of the most frequent chromosome defects. It occurs in 1-1.5 thousandth of pregnancies. Beside the present practice of genetic screening children with Down's syndrome are born alive in 49% in Hungary. Therefore it is well known amongst laymen as well. Down's syndrome can well model the difficulties of breaking bad news after childbirth. By mapping the circumstances of informing about Down's syndrome we can get an insight to the circumstances of breaking bad news in obstetric wards.

In this situation the doctors' task is much more complex than in case of informing adult patients as the recipient is not the patient. It is a special informing situation where the informed is not the patient itself. The mode of breaking news and its circumstances both have an effect on the informer and the receiver as (Ptacek and Eberhardt 1996).[10]

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The aim of the survey was to identify the factors influencing parental satisfaction in international literature and to map the circumstances of parental information in Hungarian institutions of obstetrics with the help of a questionnaire in order to evaluate the possibilities for interventions.

MATERIALS AND METHODS

After thoughtful consideration of the literature we identified those factors that, based on the present survey, would determine parental satisfaction (Table I). We took such international publications as bases that were published in the past 30 years in English in the given topic. Based on the factors influencing parental satisfaction we compiled a questionnaire with the help of the Down's team operating at the University of Pécs, Faculty of Health Sciences, the National Register for Congenital Diseases of the National Centre for Epidemiology (VRONY) and Down's Foundation. The local representatives of VRONY filled out the questionnaire between November 2005 and February 2006 based on the answers collected from the doctors of obstetric institutions. The respondents had to answer yes-no questions. In the questionnaire the questions were divided into 4 groups, the doctors of each given institution were asked about the circumstances of informing about postnatal recognition of Down's syndrome, whether they have an applied protocol, if yes, whether they use it or not. They were also asked about the support provided for the parents: whether they provide psychological support, written information materials or address list of supporting organizations. We also asked for information about the doctors' own opinion on the quality of informing, and how they evaluate their own performance.

RESULTS AND DISSCUTION

On the national level 85 institutions filled out the questionnaire, in which the number of birth in the year of the survey was 70117 (in the same year the total number of births was 95137 in Hungary). The national coverage projected on the number of births was 74%.

33% of the doctors have already felt that the mother of a newborn with Down's syndrome would expect more support than the provided, but the institutions were unable to provide. 95% of the responding institutions believed that health care professionals can contribute to the formation of a better attitude towards those with Down's syndrome (Table II).[3,12,7,9,6,13,5]

Only 6% of the responding institutions have an information protocol. However, 65% of these institutions would require having such protocol and this data shows similarity to the surveys on parental satisfaction in international reference literature (Cunningham, 1986[3], Skotko, 2005[13], Hedov, 2006[6]).

81% percent of doctors believe that the informing they perform is appropriate quality. Doctors not satisfied with their informing performance believe that diagnosis cannot be given before the results of chromosome analysis is not available (26%), or do not have up-to-date knowledge about legal, educational, social forms of treatment (15%).

Table I. Factors influencing parental satisfaction in international literature

	Cunningham et al 1984.	Quine et al 1986	Mc.Kay et al 1990	Quine et al 1994	Pain et al 1999	Hedov et al 2002	Skotko 2005	Graangraad 2006
The informer's personality	-	-	-	-	-	X	X	-
The time of informing	X	X	-	X	-	X	X	X
Concurrent informing of parents	X	-	-	-	-	X	X	X
Tone, degree of sympathy	X	X	X	-	-	X	X	-
Emphasizing positive/negative aspects	-	X	-	X	-	-	X	-
Time for asking questions	-	-	X	-	-	X	X	-
Adequate and sufficient information	X	X	X	X	-	X	X	X
Recommending support of fellows /civil organizations	X	-	-	-	-	X	X	-
Quality of the written information materials	-	-	-	-	-	-	X	-

X: the factor included in the study
- : the factor not included in the study

Table II. The answers of doctors representing the institutions to the questionnaire

Questions	Yes	No	No answer
Is it possible that the mother of a newborn with Down's syndrome would expect special help that the institution is unable to provide?	28 (33%)	53 (62%)	4 (5%)
Can health care professionals contribute to the formation of a better attitude towards those with Down's syndrome?	79 (93%)	4 (5%)	2 (2%)
Is there an information protocol in your institution?	5 (6%)	80 (94%)	0 (0%)
If not, is there a demand for it?	55 (69%)	20 (25%)	5 (6%)
Besides all your other duties can you perform the task of "informing" well enough?	69 (81%)	13 (15%)	3 (4%)
if not, what are the reasons:			
usually not required by the parents	0 (0%)	12 (92%)	1 (8%)
psychologically not prepared	1 (8%)	11 (85%)	1 (8%)
cannot feel to treat the pain of the parents	2 (15%)	10 (77%)	1 (8%)
do not have up-to-date knowledge about Down's syndrome	2 (15%)	10 (77%)	1 (8%)
do not have up-to-date knowledge about legal, educational, social form of treatment	12 (92%)	0 (0%)	1 (8%)
it is not a practice in your institution	1 (8%)	11 (85%)	1 (8%)
there is not an appropriate room in you institution	2 (15%)	8 (62%)	3 (23%)
Diagnosis cannot be given before the results of chromosome analysis is not available	12 (92%)	0 (0%)	1 (8%)
What do you consider optimal "informing" circumstances			
a separate room for such occasion	68 (80%)	5 (6%)	12 (14%)
the obstetrician alone	3 (4%)	30 (35%)	52 (61%)
the obstetrician and the paediatrician together	57 (67%)	13 (15%)	15 (18%)
the present of both parents	74 (87%)	2 (2%)	9 (11%)
the present of both parents and the baby	31 (36%)	22 (26%)	32 (38%)
right after childbirth	23 (27%)	26 (31%)	35 (42%)
1-2 days after childbirth	46 (54%)	9 (11%)	30 (35%)
on leave	2 (2%)	34 (40%)	49 (58%)
without time limit, giving time and opportunity for parents to ask all their questions	61 (72%)	7 (8%)	17 (20%)
To give the opportunity to talk about the situation more than once	73 (86%)	2 (2%)	10 (12%)
Due to the lack of time in a short and objective style	4 (5%)	44 (52%)	37 (44%)
Are there any information materials available for parents in your institution that are suitable for conveying important info about Down's syndrome?	28 (33%)	56 (66%)	1 (1%)
Are the written information materials useful?	69 (81%)	3 (4%)	13 (15%)
Is the content of written materials appropriate?	25 (29%)	2 (2%)	58 (68%)
Do parents receive any psychological or social support from a professional in your institution?	41 (48%)	20 (24%)	24 (28%)
What kind of specialist help, professional support is necessary for parents?			
in general breaking the bad news in a well elaborated manner	56 (66%)	4 (5%)	25 (29%)
help of obstetrician	43 (51%)	13 (15%)	29 (34%)
help of paediatrician	75 (88%)	1 (1%)	9 (11%)
help of psychologist	32 (38%)	14 (16%)	39 (46%)
information (brochures, leaflets, address lists)	68 (80%)	1 (1%)	16 (19%)
Do you think it would be important to introduce such training?	73 (86%)	12 (14%)	0 (0%)

87% of the responding doctors consider the circumstances of "informing" optimal if both parents are present, in the company of an obstetrician and a paediatrician (67%) in a separate room, to talk to them more than once (80%) without time limitation (86%), giving time and opportunity for parents to ask all their questions (73%), also giving the opportunity to talk about the situation more than once (86%). It should be emphasized that only 36% of the responding doctors think that presence of the child is important, that is different from the surveys on parental satisfaction in international reference literature. There one of the most important expectations of the parents was the presence of the child while being informed and they also expected the doctor to hold the baby (Skotko, 2005).[13]

Even though 81% of the responding institutions would consider information materials about Down's syndrome for parents useful, such aid was only used in

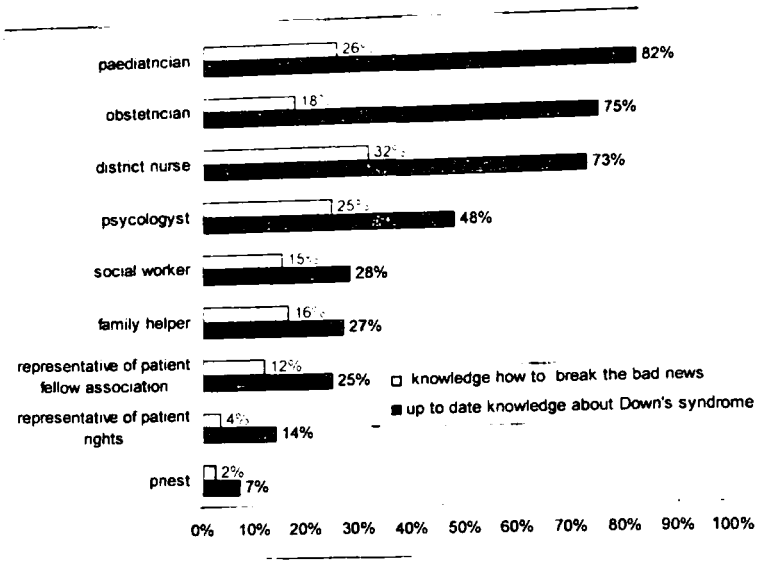

Figure 1. Education about Down's syndrome and breaking bad news in cases of each communicational participant

33% of the institutions. According to the result only 29% of these information materials were suitable for conveying important info in connection with Down's syndrome. According to certain opinion the written information materials available at present are not suitable, as they are too detailed, the parents are often fail to read them considerately due to their psychic state. In 48% of the responding institutions the parents can get some psychological help or social support. According to the respondents, in general, breaking the bad news in a well elaborated manner could be the most important part of psychological support (66%). 88% of the doctors participating in the survey parents mainly need the support from a paediatrician and information such as address list, phone numbers were thought to be the most important (80%).

The survey also touched upon the education of health care professionals about Down's syndrome, as these professionals could help to contribute to the formation of a better attitude of society towards those with Down's syndrome. Based on the results we can say that mainly paediatricians (82%), obstetricians (75%) and district nurses (73%) receive up-to-date training about Down's syndrome (Figure 1), however, they do not receive adequate help to develop their communication skills. 32% of district nurses, 25% of psychologists and 26% of the paediatricians received communication training (Figure 1). 86% of the respondents would support a possible communication training.

CONCLUSIONS

After analysing the numerical data of the survey we found that early information and support provided to parents is problematic in the majority of obstetric institutions in Hungary. The doctors do not receive communicational training on informing; in spite of that almost three quarters of them believe to perform the task of informing well enough. The international reference literature relatively often gives the exemplary guidelines to follow; it seems those are not adopted well enough in practice. Taking the interventional possibilities into account one solution could be the improving the communication skills of the concerned professionals with the help of an aimed communication training. In a significant number of the concerned institutions a need for the formation of a unified information protocol emerged, that is in harmony with the surveys dealing with international parental satisfaction.

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Clinical and endoscopical aspects in patients with drug related upper gastrointestinal bleeding

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Antithrombotic drugs and non-steroidal anti-inflammatory therapy are widely believed to be commonest iatrogenic cause of upper gastrointestinal blood loss. Given the high prevalence of both therapies in clinical practice, it is imperative that physicians weigh the potential benefits and the associated risks of these drugs. The aim of our study was to assess the clinical and endoscopical aspects among patients admitted in a medical clinic, with upper gastrointestinal bleeding. 134 bleeder patients were compared with 141 control patients admitted in the same hospital. In the bleeding group were statistical significant more risk drug users (86.5%) than in control group (44.6%) (p<0.0001), and the patients used more antitrombotics than anti-inflammatory drugs in both groups (bleeders: 69.4% vs. 28.3%; non-bleeders: 28.3% vs. 19.1%). Aspirin was most prevalent used drug alone or in various combinations in all patients, but the differences were statistical significant between the two groups regarding the proportions of consumers (alone: 54.4% vs. 22.6%, combinations: 26.1% vs. 6.3%). Antithrombotic therapy is the most common cause of non-variceal upper gastrointestinal bleeding in our settings and drug combinations increase the bleeding risks.
Key words: antithrombotic, gastrointestinal, bleeding

Usually oral antithrombotic therapy decreases ischemic risks, but this therapy may increase bleeding complications. Of the major bleeding that occurs, the largest proportion is due to the gastrointestinal hemorrhage.[1] Aspirin is among the most widely used drugs worldwide, being the mainstay of prophylactic treatment in patients with atherosclerotic disease.[3] With the development of safer antiplatelet agents such as thienopyridines and the publication of major randomized studies, the combination aspirin and clopidogrel has become more used after percutaneous coronary interventions.[1] Furthermore, antiplatelet treatment is increasingly used in combination with vitamin K antagonists in patients who have dual indications for treatment, such as those with ischemic heart disease and atrial fibrillation. Antithrombotic treatment has thus shifted towards more aggressive regimens, often involving more than one drug.[2] These therapies are widely known to lead to upper gastrointestinal bleeding. These risks may be further compounded by the ancillary use of other adjunctive medications, such as non-steroidal anti-inflammatory drugs or corticosteroids.[1] Little is known about the prevalence of drug related upper digestive bleeding in our region, due to the high prevalence of Helicobacter pylori related ulcers in our population. The aim of our study was to assess the clinical and endoscopical outcomes among patients admitted in our hospital with upper gastrointestinal bleeding.

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MATERIAL AND METHOD

We perform a retrospective study reviewing the medical records of all consecutive patients hospitalized with upper gastrointestinal bleeding (hematemesis and/or melena) within January 2006-December 2008 in 3rd Medical Clinic Târgu-Mureș. Including criteria were: patients with recent history of hematemesis (vomiting the fresh blood or "coffee ground" blood) and/or melena (confirmed by clinical testing and associated with rise in urea). Excluding criteria were: patients with anemia without overt upper gastrointestinal bleeding, patients with colonic lesions detected by colonoscopy or patients with fresh rectal bleeding (any other colonic disease), cancers and cirrhosis. We recorded demographic data, clinical aspects, symptoms, history of drug intake, endoscopic findings, smoking and alcohol habits. The hemorrhagic event was attributed to therapy if patients had used medication (low-dose

aspirin, alone or in combination with anticoagulant, clopidogrelum, other non-aspirin non-steroidal anti-inflammatory drugs) within the last 2 weeks, for more than 5 days. Consecutive patients admitted in the hospital were compared with the study group, matched for age and sex. In the study group the esophagogastroduodenoscopy was performed within 24 hours from admission, or from occurrence of bleeding signs (hematemesis or melena). Gastric and esophageal endoscopic modifications were described according to the Minimal Standard Terminology. Helicobacter pylori infection was determined using serology or biopsies. The clinical outcomes of the bleeding group were compared with the control group. The endoscopic findings were compared into patients with bleeding, divided according to drug exposure. The GraphPad program was used to analyze the data. Statistical significance was defined as $p<0.05$.

RESULTS AND DISCUSSIONS

A number of 134 patients presenting including criteria were admitted with non-variceal gastrointestinal bleeding in studied period of time. The mean age was 65.59 years in the study group and 64.07 years in the control group, comparable with mean age of patients in similar studies.[4] Clinical and demographical data are shown in Table I. From a total of 134 patients with digestive hemorrhages, 116 patients (86.5%) had a recent history of antithrombotic and/or anti-inflammatory treatments, statistical significant more frequent comparative with control patients (63 patients, 44.6%) ($p<0.0001$). The most frequent drug used was aspirin, 73 patients (54.4%) in "bleeding" group, and 32 (22.6%) in control group ($p<0.001$). The daily doses were 75mg (66 patients, 90.4%), 100mg (3 patients, 4.1%) and 250mg (4 patients, 5.4%). Aspirin was used alone in 38 patients (28.3% from all bleeder patients group), statistical significant more frequent than in control group (23 patients, 16.3% of all patients in control group); the proportion of aspirin users among bleeders was the same in Taha's study (28%).[4] Other 35 patients took minidose aspirin in different combinations: with clopidogrel (8 patients, 10.9%), oral anticoagulants or low-weight molecular heparin (9 patients, 12.3%), non-aspirin non-steroidal anti-inflammatory drugs (diclofenac, ketoprofen or indomethacin) (15 patients, 20.5%) or corticosteroids (3 patients, 4.1%). These drugs associations were more likely to appear in study group, but, only aspirin plus anticoagulants ($p=0.03$) and aspirin plus non-steroidal anti-inflammatory drugs ($p=0.0079$), reached statistical significance. Surprisingly, our study did not point out differences in non-steroidal anti-inflammatory drug consumption in bleeding group comparative with control group. The anticoagulants use was more frequent in patients with upper digestive hemorrhages comparative with patients without bleeding signs ($p=0.015$). Antithrombotic drugs were used more frequent than non-steroidal anti-inflammatory drugs: in study group, 93 patients (69.4%), comparative with 38 (28.3%) non-steroidal anti-inflammatory drugs consumers; in control group

Table I. Clinical and demographical data in study group patients

	Bleeding patients		Control patients		
	N=134	%	N=141	%	p
Male	81	60.4	85	60.2	NS
Age	65.5		64.0		
Smokers (current)	13	9.7	11	7.8	NS
Alcohol intake >10 drinks*/week	12	8.9	7	4.9	NS
Aspirin users (75-250mg/day)	73	54.4	32	22.6	<0.0001
Only low-dose aspirin	38	52.0	23	71.8	0.02
Low-dose aspirin+ clopidogrel	8	10.9	3	9.3	NS
Low-dose aspirin+ anticoagulants	9	12.3	2	6.2	0.03
Low-dose aspirin+ NSAID	15	20.5	4	12.5	0.0079
Low-dose aspirin+ steroids	3	4.1	0	0	NS
**NSAID users	21	15.6	18	12.7	NS
Anticoagulants	20	14.9	8	5.6	0.015
Steroids	2	1.49	5	3.5	NS
Chronic disease					
Arterial hypertension	61	45.5	54	38.2	NS
Ischemic heart disease	52	38.8	49	34.7	NS
Congestive heart failure	21	15.67	19	13.4	NS
Stroke	7	5.2	4	2.8	NS
Respiratory disease	17	12.6	11	7.8	NS
Renal disease	4	2.98	4	2.8	NS
Rheumatoid disease	46	34.3	27	19.1	0.0061
History of peptic ulcer	37	27.6	15	10 6	0.0004
History of bleeding	11	8.2	3	2.1	0.0274
Proton pomp inhibitor use	5	3.73	7	4 96	NS

*1 drinks= 14 ml pure alcohol
**NSAID =non-steroidal anti-inflammatory drug

Table II. Endoscopic findings in study groups patients

	Aspirin users N=73		Other NSAID users N=21		Anticoagulants users N=20		No drugs users N=18	
	N	%	N	%	N	%	N	%
Helicobacter pylori positive	22	30.1	5	23.8	6	30.0	17	94.4
Gastric ulcers or erosions	29	39.7	14	66.6	11	55.0	7	38.8
Duodenal ulcers or erosions	24	32.8	4	19.0	7	35.0	10	55.5
Gastric and duodenal ulcers or erosions	18	24.6	3	14.2	2	10.0	1	5.5
Esophagitis	44	60.67	10	47.6	9	45.0	9	50.0

the proportion was 28.3% vs.19.1%. There were no differences in age, sex, smoking habits, alcohol consumption and chronic diseases between the two groups, but rheumatoid disease occurred more frequent in bleeding patients (p=0.061). The known risk factors for major complications, history of ulcer disease and history of bleeding[1], were statistical significant more present in study group (p=0.0004, respective p=0.0274). The use of gastroprotective medication (proton pomp inhibitors) was similar into two groups.

Esophagogastroduodenoscopy was performed in all patients in study group. The endoscopic findings are shown in Table II. In our study 37.8% of patients from bleeding group were positive for Helicobacter pylori infection: 29.8% from patients with drug exposure and 94.4% from patients without drug exposure. Upper gastrointestinal bleeding was associated with gastric lesions in 61 of cases (46.2%), duodenal lesions in 45 patients (34.9%) and concomitant lesions in 24 patients (18.1%). In all medication groups, lesions were more prone to occur in gastric mucosa, while nonusers had more duodenal lesions. Esophagitis was surprisingly frequent in our study, more than a half of patients presented various esophageal changes comparative with 20% in other endoscopic series[3], with no statistical significant differences comparing medication groups with nonusers bleeding patients.

The widespread use of antithrombotic and anti-inflammatory drugs increase within last decades due to general trend of ageing world population[1], with an increasing prevalence in cardiovascular diseases and osteoraticular diseases which occur in these ages. These drugs are also widely believed to be commonest iatrogenic cause of upper gastrointestinal blood loss.[2] In our study group, drug related bleeding were common, representing more than eighty percent of all patients with non-variceal upper digestive hemorrhages (excluding patients with cancers, malformations) admitted within studied period of time in our hospital, comparable with the results in other similar study.[4] Antithrombotic drugs were more common used comparative with non-steroidal anti-inflammatory drugs in studied patients. Bleeding patients in our study were more prone to used combination drugs; low-dose aspirin was the most frequent drug used in combination with other antithrombotic or non-steroidal anti-inflammatory drugs, in more than one fourth of patients. Our results indicated that it is important to take into account the presence of esophageal lesions in patients receiving risk medication. In bleeders group, in two patients, hemorrhagic lesions were situated in lower esophagus and almost forty-five percent of all patients presented concomitant various esophageal lesions. An overall association of gastrointestinal bleeding and Helicobacter pylori infection is well documented in literature[2], and was also confirmed in our study. The use of the gastroprotection therapy, like proton pomp inhibitors, which is recommended in patients with risk factors for bleeding, was less prevalent in our study comparative with literature data.[3]

CONCLUSIONS

The main finding of our study is that antithrombotic therapy is the most common cause of upper gastrointestinal bleeding and combinations drugs are increasingly prevalent in upper digestive bleeding patients. Our results indicate that physicians need to be aware and weigh the potential risk of drug combinations against the known therapeutic benefits. Occurrence of major gastrointestinal complications is often due to the over the counter drug intake, especially non-steroidal anti-inflammatory drugs. In patients with cardiovascular prophylactic therapy with antithrombotic drugs, concomitant use of other risk medication must be carefully weighed and, often these therapies need to be supplement with gastroprotective strategies.

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Ulcerated infantile hemangiomas – a surgical approach

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Infantile hemangiomas are common vascular tumors that usually develop within the first month of life or, less commonly, are present at birth. Their evolution is characteristic, in three stages: the proliferative phase, the involution phase and the involuted phase. Most of the infantile hemangiomas are uncomplicated, but a significant number, approximately 10%, involve complications, including bleeding and ulceration. The latter represents the most frequent of all the complications that may arise in the natural evolution of this tumor.
Until recently, conservative management was the rule, the surgery being recommended only after the tumor's complete involution. This study presents the surgical approach of the ulcerated infantile hemangioma at an early stage and the results of this therapeutic strategy. Ten cases of ulcerated hemangiomas were referred to our department, over a period of 15 months. They were treated surgically, with an average follow up of 6 months postoperatively. Early excision should be considered as a first line therapy for the ulcerated hemangiomas, since plastic surgical techniques can be used more effectively and applied at younger ages than has been the custom in the past.
***Key words**: Ulcerated hemangiomas, infantile hemangiomas, surgical excision*

Infantile hemangiomas, which can be identified at 5-10% of the newborns, used to be considered "birthmarks", but unlike the majority of birthmarks, they are uniquely dynamic. At present, it is generally accepted that hemangiomas are common vascular tumors (benign) that usually develop within the first month of life or, less commonly, are present at birth.[9] Their evolution is characteristic, in three stages: the proliferative phase represents a rapid augmentation and then stabilization in 1-1,5 years, the involution phase that lasts up to 5-7 years, and the involuted phase, after the age of 10. Complete hemangioma regression is usually seen in over 50 percent of children by 5 years of age and 70 percent in children 7 years of age.[2,10] Girls are affected between two and five times as often as boys.[2-4]

Most of the infantile hemangiomas are uncomplicated, but a significant number, approximately 10%, involve complications, including bleeding, ulceration, distortion of involved tissues, obstruction of a vital structure (vision, airways) and congestive heart failure.[3] Hemangiomas can also leave residual scarring and/or permanent distortion of facial anatomic landmarks, which can be truly life threatening or cause a poor life quality due to distortion of the self image.

Spontaneous epithelial breakdown and ulcerations occurs in 5% of all cutaneous hemangiomas, more commonly in the lip or anogenital area[8], thus making this complication the most frequent of all the complications that may arise in the natural evolution of this tumor. This incidence justifies the special interest the specialists have for the best therapeutic approach that would satisfactorily solve the ulcerated infantile hemangioma. Ulcerations may appear spontaneously, after trauma, infection or as a consequence of the laser treatment of a previously "healthy" tumor.

An ulcerated hemangioma causes pain, and also increases the risk of infection and bleeding. The infant is often irritable and eats and sleeps poorly. Ulcerated hemangioma heals slowly. Treatment protocols include topical antibiotic, frequent dressing changes, other topical applications (becalpermin gel, a recombinant human platelet-derived growth factor[7,12]) pharmacologic therapy (systemic corticosteroid, interferon a-2a, bleomycin), and pulsed dye laser application. However, the most rapidly effective therapy is excision, which removes the tumor and can provide a convenient functional and cosmetic outcome. This study presents the surgical approach of the ulcerated infantile hemangioma and the results of this therapeutic strategy.

MATERIAL AND METHODS

Ten children (3 boys, 7 girls) were referred to our department with ulcerated hemangiomas, over a period of 15 months, with an average follow up of 6 months

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Table I. Patient data

Case no.	Sex	Age at surgery	Location	Size	Previous treatment
1	F	3 months	forearm	3/4 cm	local dressings
2	F	2 months	anogenital	2,5/3,5 cm	none
3	F	2 years 3 months	upper lip	2/3 cm	none
4	M	4 months	shoulder	Ø 6 cm	none
5	F	5 months	forearm	3/4 cm	bleomycin
6	M	4 months	shoulder	5/6 cm	laser CO2
7	F	6 weeks	lower lip	Ø 1,2 cm	local dressings
8	M	5 months	face	2,5/3,5 cm	systemic corticosteroids
9	F	3 months	face	2/3 cm	systemic corticosteroids
10	F	1 year 3 months	upper lip	Ø 1,5 cm	none

after the surgery. At presentation, the age of the patients ranged from 6 weeks to 2 years 3 months (average age 7 months).

Initial assessment included medical history and clinical examination, as well as some blood workups. The variables collected included patient's sex, age at surgery, tumor location, functional compromise, extent of cutaneous involvement, prior treatment, diagnostic workup, surgical technique, operative and postoperative complications, and reconstructive outcome.

Initial management, before referring to our department, was conservative in almost all cases with observation, local cleaning, a topic antimicrobial preparation and systemic antibiotics if required. One patient had a session of laser therapy. No significant improvement was noticed, concerning the ulceration or the size of the tumor.

Surgical treatment (excision) was performed under general anesthesia. Preoperatively, patients received especially local treatment, consisting of dressings with antibiotics and/or corticosteroids. Postoperative care included antibiotics, treatment for pain and swelling, local cleaning and dressings, and antibiotic prophylaxis for preventing any potential infection.

Healing was defined as the complete closure of the postoperative wound, the lack of complications, a convenient functional and cosmetic outcome, the absence of pain and the restoration of normal physiologic activities of the infant.

All patients who received surgical treatment for ulcerated hemangiomas were included in the study.

RESULTS AND DISCUSSION

As mentioned, ten patients referred to our department with ulcerated hemangiomas. Average age at surgery was 7 months. The male-to-female ratio was 1:2,33, a little lower than the average ratio reported in the literature (1:3). Patient's data are summarized in Table I. The figures (1 through 6) represent examples of three of the cases.

Considering the sites of the lesions, one patient had a hemangioma affecting the anogenital area (the right buttock, near the intergluteal region), another one had a hemangioma of the lower lip (Figure 5,6), two had hemangiomas affecting the upper lip, two presented tumors of the shoulder (Figure 3,4), two had hemangiomas affecting the face, and the last two were located on the forearm, just above the wrist.

Six out of the ten patients were referred to our department with a Doppler ultrasonography, and one of them also with magnetic resonance imaging scan, all from an outside hospital. The remaining patients were diagnosed on clinical features and surgically treated without further diagnostic workup.

The operative technique depended upon the location of the hemangioma. The purpose was to remove the entire tumor within one surgery, while leaving the smallest scars possible. In six cases, the tumor was excised and the wound was closed directly, remaining a linear scar, which represented a satisfactory result. The other four cases did not allow such an approach, and an operative technique using local flap was chosen instead.

Intraoperative bleeding was minimal and caused no technical difficulties in the excision of any of the lesions in this series. Any hemorrhage encountered was effectively controlled using the electric scalpel.

There were no major complications, one patient presenting a small hematoma evacuated at two days postoperatively. None of the cases had any recurrence of their lesions or rebound growth. Eight out of the ten cases were treated during the phase of proliferation (which turns into the involutional process at approximately 12 months of age and continues over the next 5 to 7 years)[1]. The remaining two cases, aged 15 and 27 months respectively (Figure 1,2), underwent surgery during the involuting phase.

Figure 1. Ulcerated hemangioma of the upper lip in a 27 month old child

Figure 2. Same case, postoperatively, the surgical wound being closed with direct suture

Figure 6. Same case, postoperatively. The tumor was removed and the wound closed directly

Figure 3. A 4 month old male with a large ulcerated hemangioma of the left shoulder

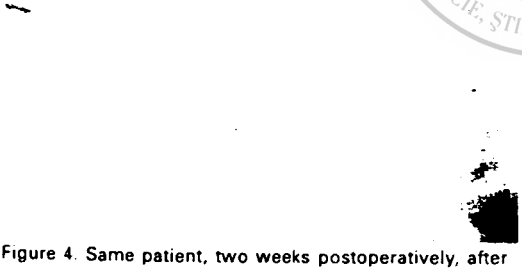

Figure 4. Same patient, two weeks postoperatively, after the removal of the sutures

Figure 5. A 6 week old female with an ulcerated hemangioma of the lower lip

The appearance of ulceration in infantile hemangiomas presents a difficult management problem. Superinfection is frequent and may lead to septicemia. Ulcerated lesions are often painful and need daily cleaning and dressings for months. Even if a hemangioma undergoes complete involution, the remaining fibrofatty skin is often atrophic.[13] The dermal layer is extremely thin and devoid of normal skin appendages. This can lead to frequent scaring and possibly marked disfigurement. It is therefore essential to treat ulcerated infantile hemangiomas as quickly and as efficiently as possible. The approach that meets both this criteria is surgical excision, at the time the ulceration occurs.

Although traditionally there has been concern that excision of proliferating infantile hemangiomas might be associated with the risk of hemorrhage and damage to vital structures associated with them (i.e., head, neck), the important benefits of this therapeutic approach cannot be overlooked. The advantages of early excision include improvement of the physiological functions affected by the ulcerated hemangioma, faster healing than after other treatment methods (1-2 weeks, instead of 1-2 months), controlled scaring that leads also to a decrease of the negative psychosocial effects associated with a cosmetically disfiguring lesion during early childhood. For some large tumors, serial excisions may be required in order to achieve a satisfactory cosmetic outcome, but it was not the case in our study.

Even though it is true that bleeding of any amount in an infant is potentially dangerous, the use of surgical instruments that cauterize while cutting lessen the risk of hemorrhage. At the same time, imaging techniques like ultrasound can help in accurately determining the flow characteristics of a vascular lesion.[5,14] Thus, the place of the vascular pedicles can be identified. This way, the surgeon may isolate the pedicle and perform a ligature, avoiding the hemorrhage.

The treatment also allows a rapid alleviation of the pain. This symptom is indeed often underestimated in infants, and the patients are not prescribed any analgesia in most of the cases. Another considerable benefit achieved by rapid healing after surgery, depending upon

the site of the hemangioma, is the restoration of feeding and other physiological functions, since infants with ulcerated hemangiomas are often irritable, feed poorly and are unable to sleep.

CONCLUSIONS

The surgical excision provides a recognized alternative to conservative management.[6,11] In our series, it has proved to be reliable, safe and effective, allowing rapid healing in all cases and a good cosmetic result, both from the surgeon and from the parents' point of view. A single operation offered definitive treatment. Therefore, early surgery should be considered as a first line therapy for the ulcerated hemangiomas, or it should be employed as soon as possible in case the pharmacologic or laser therapy show no significant improvements of the lesion. There is no need to wait for the hemangioma to involute first, since plastic surgical techniques can be used more effectively and applied at younger ages than has been the custom in the past.

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Determination of fetomaternal hemorrhage – our experience

Dana Paula Florea

The object of the study was to show the prevalence of the cases with positive fetomaternal hemorrhage (FMH) during the pregnancy and after delivery, in order to argue the necessity of introducing the evaluation tests for FMH in the laboratory practice for all the pregnant women and childwives with Rh-negative with risk of alloimmunzation. Within 2 years, there were analyzed 150 samples to which the acid elution test and ID FMH screening test were effectuated. Out of the 150 tested samples, 19 (12.6 %) had a positive result in both of the methods, out of which 5 (26.3 %) were childwives and 14 (73.6 %) were pregnant women. The analysis of the cases with a positive result suggests the fact that the risk of FMH apparition increases at the same time as the progress of the pregnancy and as the number of pregnancies in antecedents. As our results show that FMH is a frequent phenomenon during pregnancy and after delivery, we believe that its determination has a great importance for the decrease of perinatal morbidity.
***Key words**: fetomaternal hemorrhage, alloimmunization, acid elution*

FMH constitutes the physio-pathological mechanism in the alloimmunization by pregnancy and hemolytic disease of the newborn (HDN). There are several antigens and antibodies that are involved in the etiology of this disease, but one of the most severe forms is due to the anti-Rh D antibodies.

The distribution of anti-D immunoprophylaxis during pregnancy and immediately after delivery reduced the perinatal morbidity, but did not completely eliminate it. Two major causes of the anti-D immunoprophylaxis failure are:

a. the incomplete protection offered by immunoglobulin subdose, when the control of its efficacy after administration is not done and

b. the lack of prenatal immunoprophylaxis in case of risk situations.[4,11]

In Romania, the FMH evaluation is not a routine practice; the result is that a great part of the cases with alloimmunization risk remain non-diagnosed.

In this study, we have emphasized the FMH by using 2 methods on samples from pregnant women and childwives with Rh-negative. The object of the study was to show the prevalence of the cases with positive FMH during the pregnancy and after delivery, in order to argue the necessity of introducing the evaluation tests for FMH in the laboratory practice for all the Rh-negative pregnant women with risk of alloimmunzation, as well as for childwives (for the immunoprophylaxis control).

The Regional Centre for Blood Transfusion of Tirgu-Mures

MATERIAL AND METHOD

Within 2 years, there were analyzed 150 samples from Rh-negative pregnant women with an incompatible pregnancy, who came to our laboratory and Rh-negative childwives, 2 hours after the vaginal delivery of an Rh-positive baby.

The acid elution test was effectuated in parallel with the ID FMH screening test.

The method of acid elution is a cytochemistry test that identifies and quantitatively evaluates the fetal red cells on the grounds of the fact that HbF is resistant to acid elution, while HbA is not. When a thin cervical smear from a maternal sample is exposed to an acid buffer solution, the maternal red cells loose their Hb and on the smear remains only their stroma, while the fetal red cells remain intact. The smears are washed and colored with hematoxylin-eosin, and then they are microscopically examined.

The maternal red cells have a "phantom" aspect and the fetal red cells are colored in red or dark pink; up to this stage, the test may be used as a screening method.

After coloring and a first examination for the verification of color correctness and of the presence or the lack of fetal cells, the fetal cells are numbered in 25 fields where the cervical smear is very thin, then the volume of FMH is estimated by applying a calculation formula.[8,9]

The ID FMH method is a screening test based on the consumption of a specific anti-D monoclonal

antibody , indirectly measured through the method of agglutination in gel against a D-positive red cell of reference. This consumption of antibodies allows the semi-quantitative evaluation of Rh D positive red cells present in the Rh-negative blood sample. The results acquired on the researched sample are compared to those of the standard red cells in the testing kit, used in parallel.[5]

1. Distribution of cases in comparison with the time of delivery

Out of the studied group, 29 patients were childwives (19.3 %), and 121 were 1-8 months pregnant women (80.7 %).

2. Distribution of cases depending on the number of pregnancies in antecedents

From the anamnesis of the patients it results that 74 (49.3 %) were at their first pregnancy, 54 (36.0 %) at their 2nd pregnancy, 18 (12.0 %) at their 3rd pregnancy and 4 (2.7 %) at their 4th pregnancy.

3. Distribution of cases depending on the age of the pregnancy

Out of the total of pregnant women, 11 (9.2 %) were in the 2nd month of pregnancy, 11 (9.2 %) were in the 3rd month of pregnancy, 15 (12.5 %) were in the 6th month of pregnancy, 18 (15.0%) were in the 7th month of pregnancy, 21 (17.5 %) were in the 8th month of pregnancy, 8 (6.7 %) were in the 9th month of pregnancy.

RESULTS AND DISCUSSIONS

1. The test of acid elution

Out of the 150 tested samples, 19 (12.6 %) had a positive result, out of which 5 (26.3 %) were childwives and 14 (73.6 %) pregnant women.

2. The ID FMH screening test

Out of the 150 tested samples, 19 (12.6 %) had a positive result, out of which 5 (26.3 %) were childwives and 14 (73.6 %) pregnant women.

- The distribution of the positive results depending on the number of pregnancies in antecedents

Out of the 19 positive patients, 4 (21.1 %) were at their first pregnancy, 9 (16.7 %) at their 2nd pregnancy, 4 (21.1 %) at their 3rd pregnancy, and 2 (16.7 %) at their 4th pregnancy (Table I).

Table I. The distribution of the positive results depending on the number of pregnancies in antecedents

Number of Pregnancies	Negative Test	Positive Test	Total
1	70	4	74
2	45	9	54
3	14	4	18
4	2	2	4
Total	131	19	150

- The distribution of the positive results depending on the age of the pregnancy

Out of the 19 positive pregnant women, 1 (7.1 %) were in the 3rd month of pregnancy, 2 (14.3%) were in the 7th month, 9 (64.3 %) were in the 8th month, and 2 (14.3 %) were in the 9th month of pregnancy. (Table II)

Table II. The distribution of the positive results depending on the age of the pregnancy

Age of the Pregnancy	Negative Test	Positive Test	Total
2	11	0	11
3	10	1	11
4	15	0	15
5	21	0	21
6	15	0	15
7	16	2	18
8	12	9	21
9	6	2	8
Total	106	14	120

- The comparison in terms of positiveness of the 2 used techniques (Table III)

Table III. The comparison in terms of positiveness of the 2 used techniques

	ID FMH		
Acid Elution Test	Negative	Positive	Total
Negative	131	0	131
Positive	0	19	19
Total	131	19	150

The comparison of the two tests in terms of positiveness (qualitative appreciation) indicates an absolute concordance between the acid elution test and the ID FMH method.

The stage of the actual research shows that the transfer of the fetal blood in the maternal circulation through the placental breaches is an ordinary phenomenon during pregnancy and delivery. Thus, according to some authors, more than 50% of the pregnant women present more or less FMH during pregnancy and after delivery.[3,15] In our study, for a number of 150 pregnant women and childwives with an incompatible pregnancy in the Rh system, we found 12.6 % cases of positive FMH. Our percentages, lower than other similar studies may be explained by the reduced dimension of the study group.

Different studies[3,13] have proved scientifically the idea that the incidence and the proportions of FMH increase as the pregnancy progresses. Thus, 3% of the pregnancies present FMH during the 1st trimester, 12% in the 2nd trimester, 45% in the 3rd trimester of pregnancy. In our study, we have found similar results; most of the cases were discovered within the last 3 months of pregnancy. We have also noticed the fact that at the same time as the number of pregnancies increases, the number of cases of positive FMH increases, too.

CONCLUSIONS

The anti-D immunoprophylaxis is based on the correct evaluation of the FMH volume. In general, the evaluation tests of FMH have a great clinical utility because they offer the necessary information for the correct effectuation of the immunoprophylaxis and they allow the evaluation of health's condition of the conceiving product, as a result of some pathological conditions during the pregnancy.

In the present days, in Romania, the routine FMH evaluation is not performed as a consequence the prevalence of the HDN is much greater than in other countries.

As FMH is a frequent phenomenon during pregnancy and after delivery, its determination has a great importance for the decrease of perinatal morbidity.

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A comparative study of the sensibility and specificity of tru-cut needle biopsy of the breast versus excisional biopsy in the diagnosis of breast cancer

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Introduction: the diagnostic triad in breast cancer consists in clinical examination, imagistic examination (mammographic and echographic) and pathologic confirmation of malignancy. The ultrasound guided needle core biopsy is, nowadays, the preoperative standard diagnostic method. Material and method: the study assesses a group of 51 patients with breast diseases who underwent needle core biopsy, in comparison with a group of 234 patients who underwent excisional biopsy. The study analyses the comparison of the two patient groups consistent with the method's sensibility and specificity to establish a conclusive diagnosis. Results: The needle core biopsy was performed on lesions in BIRADS V stage in 43 cases (90%), BIRADS IV stage in 6 cases (9,7%), BIRADS III stage in 2 cases (2,4%), and for each lesion were sampled between 3 and 6 tissue fragments. The results were inconclusive in 3 cases of needle core biopsy, which later underwent excisional biopsy, 2 malignant and one case of benign lesion. From the cases of excisional biopsy, 23 (9,8%) presented with an inconclusive result and required follow-up or another excision. Conclusions: within our cases, the needle core biopsy had a sensibility of 93,1, superior to the incisional biopsy 90,2.

Key words: breast, cancer, biopsy, sensibility

The diagnostic triad in breast cancer consists in clinical examination, imagistic examination (mammographic and echographic) and pathologic confirmation of malignancy. The only examination which may confirm cancer existence is the pathologic analysis of the surgical sample or the tissue fragments sampled through needle core biopsy.

The proof of breast cancer is useful prior to the surgical intervention, as it impacts on its extent, if necessary, and on the following treatments. Concurrently, establishing the pathologic type offers valuable information regarding the prognostic of thedisease. For cases which undergo neoadjuvant chemotherapy, the biopsy allows tumour grading, and the establishment of important information regarding the prognostic.

The exact report of the histological type and tumour grading, prior to cytostatic treatment, is as important as in some cases chemotherapic tumour sterilization may be achieved, thus, some essential information, like hormonal receptors, regarding postoperative treatment guidance, may not be acquired.

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Biopsies may be classified as: Percutaneous needle biopsies: a. Fine needle biopsy, b. Thick needle biopsy, Tru-cut needle biopsy, c. Vacuum assisted biopsy, d. Very thick needle biopsy (tube), and surgical biopsies: a. Incisional biopsy, b. Excisional biopsy. The tru-cut needle biopsy has the following advantages[5]: it allows-the establishment of the diagnostic of breast cancer, the distinction between invasive and insitu types, the detection of immunohistochemical factors like RH and Her; it is repeatable, it has a low complication rate, high sensibility and specificity, provided the principles and indications are respected, effective cost-benefit ratio, it eliminates the false negative and positive results of the frozen section examination. Lesion marking after core needle biopsy is important, taking into account the following neoadjuvant chemotherapy, which may lead to the clinical and imagistic tumour disappearance, which will lead to difficult or impossible breast conserving surgery. The limitations of this method are provided by the echographic viewing status of the lesion. Not fulfilling these conditions lead to the acquirement of false negative results[3]. The exact indications for the different types of biopsies are still a matter of debate, but in general, fine needle aspiration is elected for breast cysts, the tru-cut needle biopsy is

selected for malignant tumours in stage BIRADS IV and V, and vacuum assisted biopsy is chosen for microcalcifications.

MATERIAL AND METHOD

The paper accomplishes a comparative study on the efficiency of incisional biopsy versus ultrasound guided tru-cut needle biopsy on the cases within the First Surgical Clinic, The Mures County Clinical Hospital, Targu-Mures; during 2004-2008.

All cases that underwent core needle biopsy also experienced imagistic examination, mammographic and/or echographic, with imagistic BIRADS staging, which allows a better result gradation. The BIRADS classification consists of 7 classes and is suitable for both mammographic and echographic examination:

0- requires additional examinations,
I- normal aspect,
II- positive benign lesion,
III- probably benign lesion,
IV- lesion with suspicion of malignancy,
V- lesion with certain characteristics of malignancy,
VI- malignant lesion, also confirmed by pathologic examination through prior biopsy.

The breast biopsy was performed with a spring loaded PRO-MAG ™ ULTRA biopsy gun, with fixed range of 22 mm. The biopsy needles we used are of 14-16 G, we also used coaxial needle introducers, which passes through the skin, near the lesion. These coaxial needle introducers have two great advantages: it allows sampling several tissue fragments through only one percutaneous pathway and it prevents seeding across the biopsy needle passage[4].

The principles of breast biopsy are: to visualize properly the lesion, to always visualize the needle during the procedure, local compression for 10 minutes after needle extraction, interpreting the results in consistence with the imagistic and clinical data, and repeating the biopsy or changing the technique for the inconclusive data.

All surgical interventions of tumour excision, breast conserving surgery and lumpectomies, for which we did not have prior pathological report were included within the excisional biopsy.

Cases of total mastectomies with or without lymph node dissection were excluded from this lot.

RESULTS AND DISCUSSIONS

A number of 563 women underwent surgical interventions or biopsies during this period of time. The percutaneous breast biopsy was performed in 51 cases, representing 9,05% of all the procedures performed on the breast. The excisional biopsy was performed in 234 cases, representing 41,5% of all the breast procedures.

Needle core biopsy was performed on patients who were considered in the stages BIRADS III-V, according to the mammographic and echografic classification. Most cases, 43 cases (90%), are included in BIRADS V stage, with high suspicion of malignancy, in general with large tumour masses and who underwent preoperative neoadjuvant chemotherapy. The tru-cut needle biopsy confirmed the diagnostic of breast cancer in all the cases, with an accuracy of 100%.

A number of 6 cases (11,76%) with percutaneous biopsy were classified in stage BIRADS IV, which includes the potentially malignant lesions, which would benefit of surgical intervention as the first therapeutical step.

In a number of 3 cases (50%), the results were inconclusive, requiring excisional biopsy, with a benign lesion, fibroadenoma, in one case, and malignant lesions in 2 cases. In the two cases of malignant process, the lesion was hardly visible on ultrasound examination, but clear on the mammogram; but the stereotactic biopsy was not available, and ultrasound approach was considered.

We performed needle core biopsy in 2 cases (3,92%), staged as BIRADS II, with typical aspect of fibroadenoma, the diagnostic being confirmed on the pathological report, too. The preoperative examination was not always complete regarding the excisional biopsies, also, the study being performed on the last five years, the echography and mammography results were not staged in the BIRADS classification, thus, only a global comparison may be considered.

Considering the excisional biopsies results, there were a number of 211 cases with conclusive results, with 115 (51%) benign lesions and 106 (49%) breast cancers. In 23 cases (9,8%) we had inconclusive results, which required the case's reevaluation or a second excision. The sensibility of the two diagnostic methods is evaluated through the contingency test $2x2^2$. The specificity of the two diagnostic methods is 100%; the false positive results are not due to the sampling technique but due to some errors during the pathological examination. We have not encountered false positive results. Concerning the breast needle core biopsy, the method's sensibility was determined as 94,11%, and for the excisional biopsy as 90,11%. The specialty literature mentions a sensibility of nearly 100% for excisional biopsy, which is characteristic for the excision of easily palpable lesions[1].

CONCLUSIONS

The study of the sensibility and specificity for ultrasound guided percutaneous biopsy versus excisional biopsy on the analyzed lot reveals sensibly equal results, with a slightly increased sensibility for the minimal invasive procedure. The study reveals the applicability and the security of the percutaneous biopsy, which, we consider, it should represent a first step for breast tumour diagnosis, which are visible on echography. For lesions which are not visible on echography, the pathological diagnosis is performed based on stereotactic biopsy and excisional biopsy, with or without preoperative marking[5]. It would be important to perform comparative evaluations of these methods, with all the cases included within the BIRADS

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The role of fetal fibronectin determination in premature birth prediction

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The objective is to determine the accuracy of fetal fibronectin which can predict birth in women with symptoms of premature labor.
Material and method: We performed a prospective epidemiological study including 48 pregnant women with symptoms of premature labor by collecting cervico-vaginal secretion samples during 24 - 34 week of pregnancy.
Results: The obtained values showed a higher sensitivity of the test for cases where birth occurred within 7 days (75%), although fibronectin determination showed an improved specificity value, being of 75, 68% in the first 7 days, 76.47% in the first 14 days and 78.13% in the first 21 days of determination. The positive predictive value was 25% in the first 7 days, 33.33% in the first 14 days and 41.67% in the first 21 days. We obtained the best value parameter for the negative predictive value: 96.55% in first 7 days.
Conclusions: The determination of fetal fibronectin is particularly useful in the elimination of pregnant women from the risk category of premature birth; fetal fibronectin can be used for the prediction of premature birth, especially in those occurring within 7 days of testing.
Keywords: premature birth, fetal fibronectin

Premature labor is defined as a labor triggered before the expiration of the 37th week of gestation and that is the major cause of premature birth. Patients showing signs and symptoms of premature labor are a real challenge for the clinician. Several studies have shown that over 70% of patients assessed as presenting the risk of premature birth do no give birth in the next 7 days and that nearly 40% of women, whether they are subjected to a treatment or not, give birth in due course. On the other hand, approximately 20% of women who are not considered to be exposed to the risk of premature birth, give birth prematurely.[9]

Early identification of premature labor is difficult because symptoms can be alleviated or may occur frequently in normal pregnancy too. Women diagnosed to present the risk of premature birth are subjected to various therapeutic actions including physical rest, hospitalization, tocolysis and administration of antenatal corticoids. These interventions are associated with both maternal risks as well as high costs and many times they generate unwanted social, material, psychological effects upon both the mother and the family. Therefore, the application of an as accurate evaluation method as possible to discover the risk of premature birth in symptomatic women, would help to identify those who would benefit the most from various clinical treatments and would help in reducing the number of these interventions in those who give birth at the proper time.

Fetal fibronectin is a glycoprotein with high molecular weight, produced in 20 molecular isoforms by a variety of cells, including hepatocytes, malignant cells, fibroblasts endothelial cells and amnion. It is present in the maternal blood and amniotic fluid in high concentrations and it is involved in intercelular adhesion, in relation to implantation and maintenance of placenta adhesion in the decidua. Fetal fibronectin is normally found in cervicovaginal fluids during 16 - 20 weeks of pregnancy, reappearing before birth. It is considered that the reappearance of fetal fibronectin is the physiological signal that labor was triggered [6,9].

The objective of the paper is to determine the accuracy of fetal fibronectin, which may predict premature birth in women with symptoms of premature labor.

MATERIAL AND METHOD

We conducted a prospective epidemiological study including 48 pregnant women with symptoms of premature labor, who were admitted to the Obstetrics and Gynecology Clinic 2, Tg. -Mureş during July-

Obstetrics and Ginecology Clinic 2, University of Medicine and Pharmacy Târgu-Mureş

Table I.

	birth < 7 zile	birth > 7 zile	birth < 14 zile	birth > 14 zile	birth < 21 zile	birth > 21 zile
fFN +	3	9	4	8	5	7
fFN -	1	28	3	26	4	25
Se	75,00%		57,14%		55,56%	
Sp	75,68%		76,47%		78,13%	
VPP	25,00%		33,33%		41,67%	
VPN	96,55%		89,66%		86,21%	

December 2008. We collected cervico-vaginal secretion samples from the bottom of the posterior vaginal sac by the aid of a tampon. Their age was between 24 - 34 weeks of pregnancy, calculated from the last date of normal menstruation and confirmed by the measurement of the craniocaudal length during the first trimester echographic examination. There was no sampling in pregnant women threatened of premature birth to demonstrate that dilatation was over 3 cm, membranes were broken or patients had vaginal bleeding. We excluded 7 patients from our study who gave birth by Caesarean section, or who gave birth in an interval of less than 21 days of sampling.

RESULTS

Out of the total 41 patients who met the criteria of inclusion, 12 of them tested positive and 29 negative at the identification of fetal fibronectin. Out of the total 41 patients included in the study, 14 gave birth prematurely and 27 at term. The average age of pregnancy at birth was 35.9 weeks in those who tested positive for fibronectin and 38.1 weeks for those who tested negative. Regarding the use of fetal fibronectin determination from cervical-vaginal secretions as a diagnostic test for premature birth, we evaluated several parameters, namely sensitivity, specificity, positive predictive value and negative predictive value attaining the following results:

The obtained values showed a higher sensitivity of the test for cases where birth occurred within 7 days after sampling (75%), while for those that took place after 14 and 21 days, these values were lower (57.14% and 55.56%), this parameter indicates the proportion of subjects who tested positive. Although the determination of fibronectin according to our study has a better specificity, meaning that it indicates with a greater accuracy those who does not have the disease, the proportion of subjects with negative test who gave birth prematurely was 75, 68% in first 7 days, 76.47% during the first 14 days and 78.13% in the first 21 days of determination. The positive predictive value, thus the probability of a subject who tested positive to be diseased was very low. The proportion of those who gave birth prematurely out of the total who tested positive was 25% in first 7 days, 33.33% in first 14 days and 41.67% in the first 21 days. The parameter that presented the best values was the negative predictive value, the probability of a negative test to be free of disease. In this case the proportion of persons who did not give birth prematurely out of all the total who tested negative was 96.55% in the first 7 days, then decreasing to 89.66% at 14 days and 86.21% at 21 days.

DISCUSSIONS

Similar results were obtained in similar studies. Thus, Leeson et al and Peaceman et al demonstrated that although the positive tests of fetal fibronectin were associated with premature birth, negative results have more importance in predicting the fact that premature birth will not take place [5,7]. Joffe et al demonstrated the importance of determining fetal fibronectin to reduce the duration of hospitalization and the prescription of tocolytic agents, but at the same time the administration of corticosteroids increases. [4]. Parry et al also noticed that the duration of hospitalization in women tested for fetal fibronectin decreased, reducing it from 3.8 days to 2.9 after sampling.[8] Goldenberg et al showed in a study performed in 1996 that a positive fetal fibronectin at 24 weeks of gestation would be an important factor for predicting premature birth. In a study performed in 2000 he indicated that the detection of early fetal fibronectin at the 18-22 weeks of gestation would be more predictive for premature birth [1,2]. Honest et al showed in a meta analysis which included 28 studies performed in symptomatic pregnant women and 40 studies in asymptomatic ones, including a total number of 26,876 women, that fetal cervical-vaginal fibronectin is the most accurate test for the prediction of premature birth in the first 7-10 days of sampling, among women who present the risk of premature birth before advanced cervix dilation [3]

CONCLUSIONS

1. The determination of fetal fibronectin is particularly useful in the exclusion of pregnant women from the risk category of premature birth.

2. Fetal fibronectin can be used for the prediction of premature birth, especially in those cases that occur within 7 days of sampling.

3. The determination of fetal fibronectin can be beneficial to pregnant women; obtaining negative values this can reduce the excessive unnecessary interventions in them.

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Sevoflurane postconditioning associated cardioprotection is eliminated by high-dose ketamine in rat hearts in vivo

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Sevoflurane is the volatile anesthetic able to elicit cardioprotective effects similar to ischemic pre- and postconditioning. Ketamine (K) was reported to abolish the anti-infarct protection of ischemic preconditioning. The present study investigated the effects of Ketamine (80 and 20 mg/kbw) on Sevoflurane induced cardioprotection. High- or low-dose Ketamine anesthetized rats subjected to 30 min of regional ischemia and 120 min reperfusion were randomized to receive: (i) no additional intervention (Control), (ii) Sevoflurane preconditioning (SevoPre) achieved by 2 episodes of 5 min Sevoflurane administration followed by 10 min washout, (iii) Sevoflurane postconditioning (SevoPost) achieved by 5 min of volatile agent administration at the beginning of reperfusion. Anti-necrotic effect was assessed by the triphenyl tetrazolium staining. Cardioprotection elicited by Sevoflurane preconditioning was not affected by either Ketamine doses (H/L); the infarcted area was significant lower (28±5% and 24±6%, respectively; mean±S.E.) as compared to the Control group (51±8%, p<0.05). Postconditioning with Sevoflurane preserved the protective effect in the presence of low-dose but was blunted when high-dose Ketamine was used (31±4% and 47±10%, respectively). In the experimental model of regional ischemia in rats, Ketamine in high dose abolished the anti-infarct protection of Sevoflurane postconditioning, but not the protective effects of anesthetic preconditioning.
Key words: Sevoflurane, preconditioning, post-conditioning, cardioprotection

Myocardial ischemia/reperfusion injury is a leading cause of morbidity and mortality and therapeutic strategies aimed at limiting cardiomyocyte death during the postischemic reperfusion and in the perioperative settings are nowadays extensively studied. Different mechanical and pharmacological strategies applied before (preconditioning) or after a prolonged ischemic insult (postconditioning) are able to elicit powerful cardioprotection via complex, partially elucidated signal transduction cascades.[3] Volatile anesthetics effectively elicit pharmacological pre- and postconditioning and are able to protect the heart and other vital organs. The degree of protection is comparable to the one elicited by their widely investigated counterparts, ischemic pre- and postconditioning.[4,9] However, several papers reported that ischemic preconditioning associated cardioprotection was blunted in experimental settings when ketamine was used.[6,5,1] The present study was aimed at investigating the effects of ketamine on cardioprotection associated with sevoflurane pre-and postconditioning.

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MATERIAL AND METHODS

All investigations conformed to the Guide for the Care and Use of Laboratory Animals (US National Institute of Health no. 85-23, revised 1996) and the regulations of the Romanian Law on the Protection of Animals and were approved by the Institutional Committee of Ethics and Deontology of our University.

Animal Preparation.

Male Sprague-Dawley rats (250-350 g body weight) were anaesthetized with ketamine (20 or 80 mg/kg) intraperitoneally. After tracheal intubation the right carotid artery was cannulated for measurement of aortic pressure throughout the experiment. A standard limb lead II electrocardiogram and rectal temperature were also continuously recorded. After a left-sided thoracotomy and pericardiotomy a ligature (5-0 prolene) was passed below the left descending coronary artery serving the left anterior wall. The

ends of the suture were threaded through a propylene tube to form a snare. The coronary artery was occluded by tightening the snare. Successful coronary artery occlusion was verified by the appearance of epicardial cyanosis. At the end of the surgical preparation, after placing the ligature, rats were allowed to stabilize up to 45 min according to the experimental protocols. All animals underwent 30 minutes of ischemia and 120 minutes of reperfusion.

Infarct Size Studies. At the end of the 2 h-reperfusion period, the animals were euthanized, the suture around the left coronary artery was reoccluded and Evans blue dye (Sigma Chemicals) was injected into the left ventricle via the left carotid artery cannula and allowed to diffuse for approximately 1 minute. This step identifies the area at risk as the red area of myocardium left unstained by the blue dye. Then the heart was excised, the right ventricle carefully removed, the left ventricle weighed and freezed at -20°C and transversely sectioned in 2 mm slices from apex to base. These slices were incubated in triphenyltetrazolium chloride (TTC) solution to identify the area of necrosis (AN) as the pale area of myocardium left unstained by the TTC. The ratio of the area of necrosis (AN) to area at risk (AR) defines the infarct size.

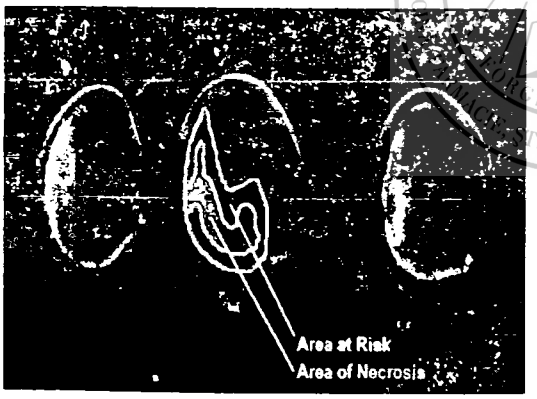

Figure 1. Triphenyltetrazolium chloride (TTC) staining for infarct size assessment in vivo. Area of necrosis (AN) appears pale whereas the area at risk (AR) is brick red in the presence of TTC, normal area is blue (by the blue dye)

Experimental protocol. Animals (n = 6 - 8/group) anesthetized with either low-dose (20 mg/bw, K20) or high-dose (80 mg/bw, K80) ketamine were randomly assigned to one of the following groups:

1) Control group (Ctrl, no additional intervention);

2) Sevoflurane preconditioning (SevoPre, preconditioning by two episodes of 5 min sevoflurane 2.5% interspersed with two 10 min washout episodes)

3) Sevoflurane postconditioning (SevoPost, 5 min postconditioning with sevoflurane 2.5% starting 3 min prior to and administered for 2 min within reperfusion).

Statistical Analysis. Data are presented as means ± S.E. Difference in the relationship between infarct size and area at risk were evaluated by one-way ANOVA and post hoc Tukey's test (Graphpad Prism v 4.0 software). A p value < 0.05 was considered to be statistically significant.

RESULTS AND DISCUSSIONS

The infarct sizes expressed as the ratios of the areas of necrosis (AN) to the areas at risk (AR) are presented in Table 1 (means ± S.E.) and in the correspondent Figure 1.

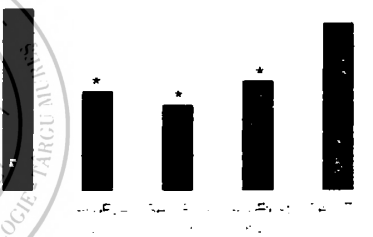

Figure 2. Infarct size (AN/AR) in the experimental groups (* p < 0.05 vs Control)
(AN = Area of Necrosis, AR = Area at Risk)

Cardioprotection elicited by Sevoflurane preconditioning was not affected by the Ketamine dosage (80 or 20 mg/kbw); the infarcted area was significant lower (28± 5% and 24±6%, respectively) as compared to the Control group (51±8%, p<0.05). Postconditioning with Sevoflurane was able to decrease infarct size only when Ketamine in low dose was used, but the cardioprotective effect of the volatile anesthetic was abolished in the case of high-dose Ketamine group (31±4% and 47±10%, respectively).

In our hands the postconditioning protocol was not able to elicit cardioprotection in the presence of high-dose Ketamine. These data are intriguing since postconditioning with Sevoflurane recapitulated a similar protocol used by Obal et al.[8] in the same experimental settings (in vivo rat hearts). However, an

Table 1. Infarct sizes in the experimental groups. (K20 - 20mg/kbw Ketamine, K80 - 80mg/kbw Ketamine)

Group	Control K20	Control K80	SevoPre K20	SevoPre K80	SevoPost K20	SevoPost K80
Infarct size (AN/AR)	46% ±6%	51% ±8%	28% ±5%	24% ±6%	31% ±4%	47% ±10%

important difference in their model was that background anesthesia was maintained by continuously alpha-chloralose infusion. However, in the presence of low-dose ketamine the Sevoflurane postconditioning related cardioprotection was not blunted. In the in vivo rabbit hearts, racemic ketamine eliminated not only the short term protection elicited by ischemic preconditioning but it also blocked cardioprotection induced by ischemic late preconditioning.[7] Interestingly, in humans an opposite effect for Ketamine, i.e. the capability to induce preconditioning in isolated atria, was described.[2] Clearly further studies are needed to characterize interferences between anesthetics in clinical settings.

CONCLUSIONS

In the experimental model of regional ischemia in rats, Ketamine in high dose abolished the anti-infarct protective effect of Sevoflurane postconditioning, but not protection elicited by anesthetic preconditioning. The dose-dependent interference of Ketamine with cardioprotection elicited by different protocols of anesthetic conditioning may be relevant in the clinical settings when their association is considered. We are currently investigating the effects of different anesthetic regimens used during the surgical preparation and maintenance on cardioprotection associated with anesthetic pre-and postconditioning protocols.

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Coexpression of c-kit and stem cell factor in head and neck cancer

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The c-kit receptor and its ligand, the stem cell factor (kitLG, SCF) participate in the regulation of several cellular processes. Their increased expression and mutations, respectively, generally associating enhanced malignant tumor behaviour have been described for various malignant tumors. The tumor, if produces both the receptor and its ligand at increased pace, may result in the formation of an auto- and paracrine loop that promotes cell proliferation. In addition, it may also stimulate the pro-angiogenesis activity of c-kit positive mast cells that play a crucial role in tumor growth. The aim of this study was to demonstrate this phenomenon, if any in the squamous cell head and neck cancers. Real time reverse transcription-polymerase chain reactions, Western blot analyses and immuno-histochemical examinations were performed on surgical samples deriving from 41 head and neck carcinomas, their metastases and peritumoral mucosa and on samples from 19 benign lesions (intact mucosa and lymph nodes), total n=60.

The coordinated expression patterns of the two markers appeared at RNA level both in the tumor samples and in the tumor free ones. Significant differences were observed in the expression of both factors between the intact mucosa and the primary and metastatic hypopharyngeal tumor as well as between the primary laryngeal and primary hypopharyngeal tumor. In the laryngeal tumors and their metastases both kitLG and c-kit showed decreased expression. This phenomenon was not present in the hypopharyngeal tumors. Results could be confirmed at protein level. A co-ordinative expression of c-kit and kitLG was seen in the intact mucosa: the same but of lesser extent was present in the laryngeal tumors, whereas in the pharyngeal tumors the decrease was more pronounced. By immuno-histochemistry the kit and kitLG positive reactions were localized to the mast cells, either specimens from the tumor, intact mucosa or tonsilla were concerned. In the squamous carcinoma cells no positive immuno-histochemical reaction could be detected. It is presumed that for the co-expression of local kitLG and c-kit, the mast cells are responsible. Mast cell migration to the tumor site and their subsequent activation may be a prerequisite for their promoting effect on tumors.

***Key words**: c-kit, stem cell factor, head and neck cancer, mast cell*

The c-kit transmembrane tyrosine-kinase receptor activated by its ligand, the stem cell factor (SCF, kitLG), mediates several cellular processes such as: gametogenesis, melanogenesis, haematopoesis and angiogenesis and affect cell proliferation, differentiation, adhesion and chemotaxis. The key role of c-kit mutation was verified in the pathogenesis of many tumors and the co-expression of c-kit and kitLG was described in some cases presuming an autocrine and paracrine effect of the ligand/receptor system on tumor behaviour.

Besides head and neck tumors the phenomenon of co-expression of c-kit and kitLG and their correlations with tumor characteristics and course of disease were only described in nasopharyngeal tumors. The aforementioned role of the c-kit/kitLG system in angiogenesis has its central scene in the mast cell environment of tumor stroma that has been extensively studied. The mast cells themselves are also c-kit positive. It is presumed that the kitLG producing tumor may facilitate angiogenesis required for tumor growth by its effect on the proliferation and migration of mast cells. In this study we wished to demonstrate whether this mechanism does take place in head and neck cancers or not.

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MATERIAL AND METHOD

Surgical preparations from 60 patients were studied. Samples derived from 41 squamous cell head and neck tumors (34 males and 7 females, mean age 52 years) of which 13 were laryngeal, 27 hypopharyngeal and 1 base of tongue squamous cell cancers and from their metastases, from intact lymph nodes, intact mucosa as well as from the intact mucosa, tonsillae and lymph nodes of 19 patient operated on for other reasons. Tumor distribution according to histological grades: 7-grade 1, 9 grade 2, 14 grade3 and 7 grade unknown. The TNM classification is shown in Table 1. With 4 cancers the TNM classification was unknown.

Table I

	N0	N1	N2
T1	2	1	0
T2	3	5	5
T3	3	4	5
T4	1	3	5

RNA isolation

Total RNA was extracted under RNase-free conditions. Frozen tissue was lysed in TRI Reagent (Sigma, St. Louis, MO). RNA was extracted according to the TRI Reagent protocol; an average of 40 mg of total RNA was isolated per sample. Total RNA yield and purity was assessed spectrophotometrically. All samples had A260/A280 ratios of 1.8-2.0.

Quantitative real-time RT-PCR

Isolated total RNA was used as a template for the production of first strand cDNA using random hexamers (Promega, Madison, WI). Relative quantification of target mRNA was performed by a TaqMan real-time RT-PCR assay on an ABI PRISM 7000 Sequence Detector (Applied Biosystems, Foster City, CA). Real-time PCRs were performed following the supplier's instructions (ABI), in the ABI-TaqMan™ Master Mix (without uracyl-N-glycosylase UNG) with primers at 300 nM each, probe at 200 nM, and 2 µl of the reverse transcription reaction respectively, in 25 µl volume reactions. Pairs of primers and TaqMan probes were designed by ABI (TaqMan® Gene Expression Assays). All reactions were set up in triplicates. Analyses of real-time quantitative PCR data were performed using the comparative threshold cycle [Ct] method. Therefore, the target quantity Xtarget at threshold cycle Ct is given by: Xtarget=2-DDCt.

Western blotting

For protein isolation samples were homogenized in a buffer containing 10 mM Tris-HCl pH 8.0, 10 mg/ml leupeptin, 0.5 mM EGTA, 2% NaF, 1% Triton-X 100, 25 mM phenyl-methyl-sulphonyl-fluoride and 2% Na-orthovanadate. Debris was removed by centrifugation, and protein yield was assessed by spectrophotometry. Then,10 µg protein samples were loaded on precast Ready Gels (Bio-Rad, Hercules, CA, USA). Gels were blotted to PVDF membranes (Bio-Rad), blocked, and blots were probed with rabbit anti-human c-kit(1:200), or rabbit anti-human kitLG (1:1000), or rat anti-human beta-tubulin (1:4000, all from AbD Serotec) antibodies, as stated. Blots were washed, and secondary goat anti-rabbit IgG-HRP (1:10 000) or rabbit-anti rat IgG κ & λ chain-HRP antibodies (1:10 000, all from Sigma-Aldrich) were applied, as appropriate. After subsequent washes, immunoreactive bands were visualized by the ECL Plus Western blotting Detection System (GE Healthcare – Amersham, Piscataway,NJ, USA). Image analysis was done using a Fluorchem 8000 image analysis platform (Alpha Innotech, San Leandro, CA, USA) and the ChemiImager 5500 image analysis software package (Alpha Innotech). Specific band size was determined with help of a Full Range Rainbow Molecular Weight Marker (GE Healthcare – Amersham).

Immuno-histochemistry

Resected specimens from patients were longitudinally sliced, fixed in 10 % formalin and embedded in paraffin. Representative sections were cut at 4 µm and stained with hematoxylin-eosin for routine histological diagnosis.

Sections for immunohistochemistry (IHC) were deparaffinized in xylene, rehydrated in graded ethanol, and incubated in 0,3 % H_2O_2 solution in methanol for 15 min to block endogeneous peroxidases. Antigen retrieval was achieved of boiling sections for 15 min in 0,01 M citrate buffer, pH 6.0. Nonspecific binding sites were blocked with background reducing, antibody diluent (Dako, A/S, Glostrup, Denmark) and by incubating sections in Tris-buffered NaCl solution with Tween 20, pH 7,6. The slides were rinsed three times with Tris-buffered NaCl solution with Tween 20, pH 7,6 for 5 min and incubated at room temperature for 60 min with a polyclonal rabbit anti-human c-kit (CD117) antibody (1:50; Dako, A/S, Glostrup, Denmark) and over night 4°C with a polyclonal gout anti-human kitLG antibody (1:200; R&D Systems Europe, Ltd. Abingdon, UK). After which the slides were again washed with Tris-buffered NaCl with Tween 20, pH 7,6 for 10 min and detection was performed with a commercial peroxidase labelled ready to use (RTU) Vectastain universal Quick kit (Vector Laboratories, Inc. Burlingame, CA, USA) according to the manufacturer's instructions. 3-Amino-9-ethylcarbazole (Vector Laboratories, Burlingame, CA, USA) and DAB+Liquid (Dako, A/S, Glostrup, Denmark) were used as chromogen and slides were counterstained with Mayer's hematoxylin. The negative control slides for IHCs were performed routinely: (1) primary antibodies were substituted with the isotype-matched control antibodies; (2) secondary antibody was substituted with Tris-buffered NaCl solution pH 7,6. The sections from human tonsilla were used as positive controls for IHCs of primary antibodies tested.

RESULTS

The expression of the two genes was demonstrated in all specimens. The highest expression was measured in the intact epithelium. Considering this significant differences were observed in the expression of both factors between the intact mucosa and the primary and metastatic hypopharyngeal tumor as well as between the primary laryngeal and primary hypopharyngeal tumor.

In the laryngeal tumors and their metastases both kitLG and c-kit showed decreased expression. This phenomenon is not present in the hypopharyngeal tumors (Figure 1).

No expression difference of either gene was detected between the intact and the peritumoral mucosa (Figure 2).

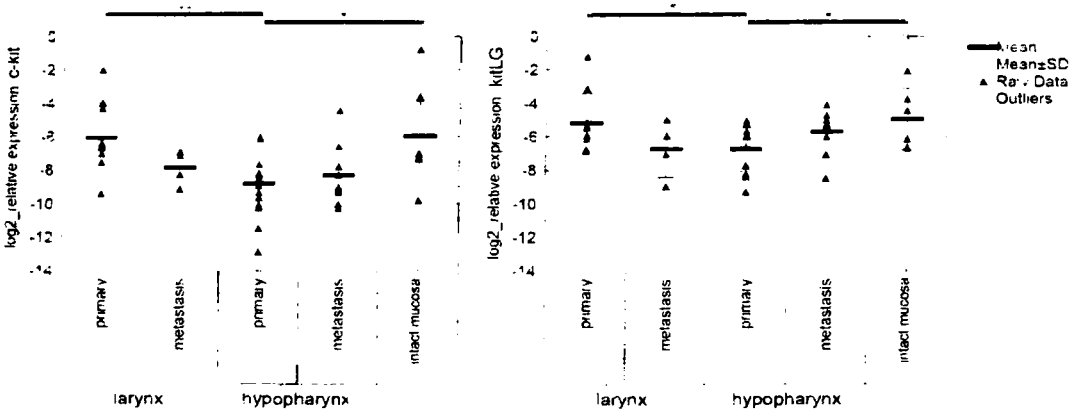


Figure 1. Relative expression of c-kit (a) and kitLG (b) in primary tumors and their metastases of larynx and hypopharynx compared to intact mucosa.
Significant differences were observed between the intact mucosa and primary hypopharyngeal tumor tissue as well as between the primary tumor tissues derived from larynx and hypopharynx. The statistical analysis was performed by the one-way ANOVA followed by the Tukey-HSD post-hoc test. (Asterisks indicate the level of significance between the respective groups: * p<0,05, ** p<0,01)

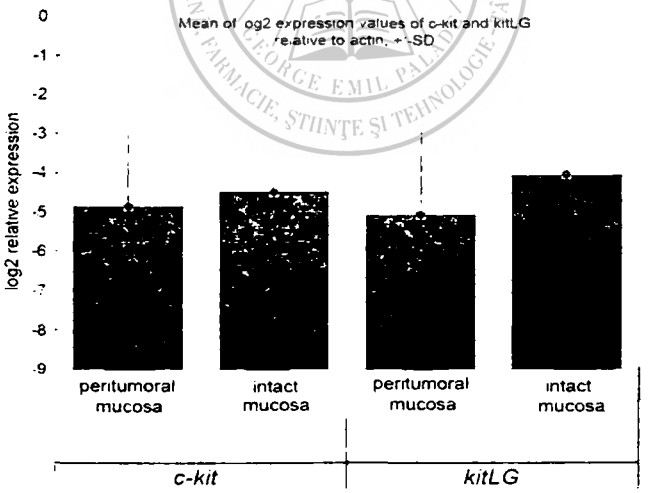


Figure 2. Comparison of expression of kit and kitLG in intact and peritumoral mucosa specimens
There are not any differences between the healthy mucosa and the peritumoral adjacent mucosa in respect to relative expression of c-kit and kitLG.
Relative expression data (converted to log2 scale) were calculated by 2(-dCT) method using ACTIN as internal control. Mean, +/-SD

Figure 3 demonstrates the expression values measured in the primary laryngeal and hypopharyngeal tumors and in their metastases: in three of the four metastatic laryngeal tumors both the c-kit and the kitLG expression decreased in the metastases compared to their corresponding primaries. However, in the hypopharyngeal ones no tendency could be detected in the course of gene expression.

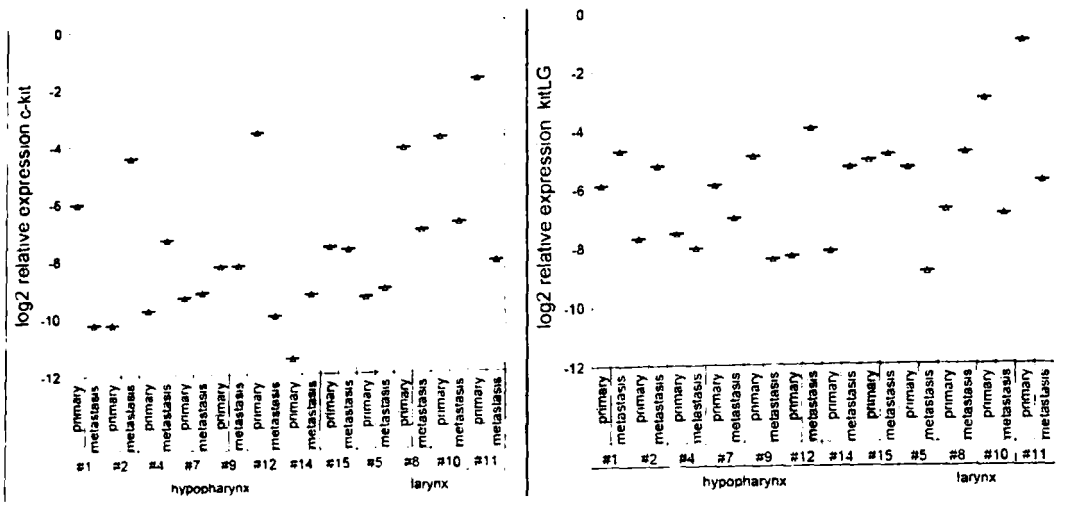


Figure 3. Individual alterations of c-kit and kitLG expression between the primary tumors and their metastatic derivates

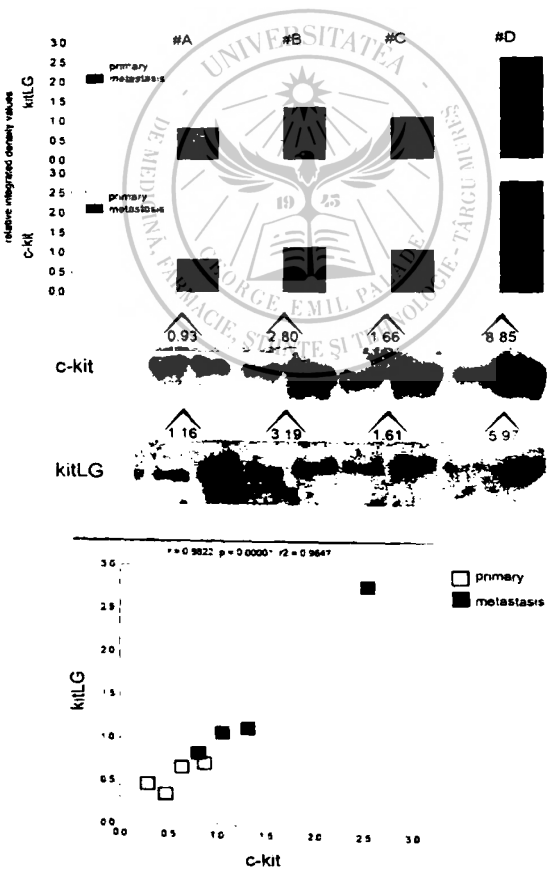
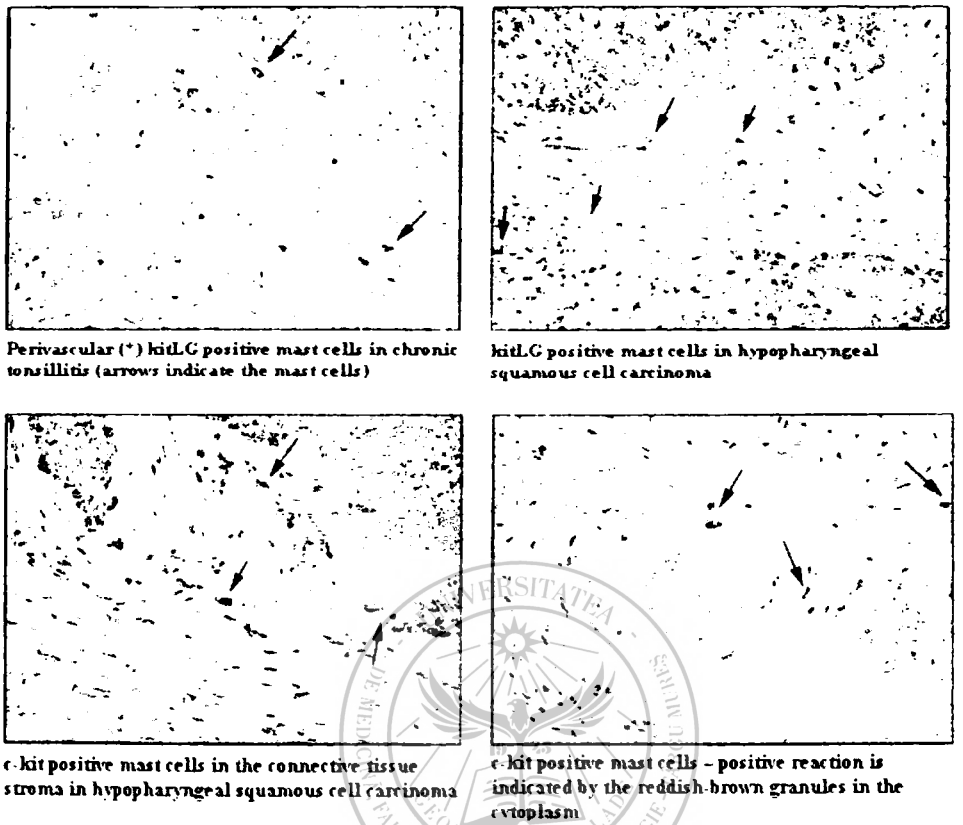


Figure 4. Individual alterations in expression of kit and kitLG at protein level determined by Western blot. The bands are quantified by densitometry. The diagrams show the integrated density values (x1000) of kit and kitLG in primary and metastatic tumor tissues of four patients. The numbers above the images indicate how many times the relative change of expression in the metastasis is higher than that in the respective primary tumor tissue



Perivascular (*) kitLG positive mast cells in chronic tonsillitis (arrows indicate the mast cells)

kitLG positive mast cells in hypopharyngeal squamous cell carcinoma

c-kit positive mast cells in the connective tissue stroma in hypopharyngeal squamous cell carcinoma

c-kit positive mast cells – positive reaction is indicated by the reddish-brown granules in the cytoplasm

Figure 5. Representative areas of immuno-histochemical reactions in head and neck cancer and chronic tonsillitis.

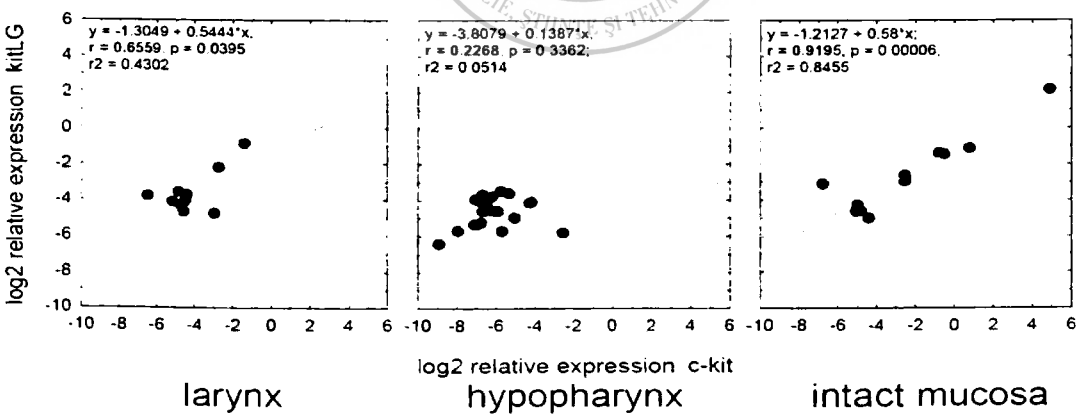


Figure 6. The coordinative expression of kit and kitLG is partly (larynx), or almost completely (hypopharynx) diminished in tumor tissues compared to control mucosal tissues. Linear fitting with regression bands indicating the 95% confidence intervals

Results were confirmed at protein level (Figure 4): The very amounts of kit and kitLG proteins measured in the primaries and their metastases, respectively confirm the co-expression experienced at mRNA level.

By immuno-histochemistry the kit and kitLG positive reactions were localized to the mast cells, either specimens from the tumor, intact mucosa or tonsilla were concerned. In the squamous carcinoma cells no positive immuno-histochemical reaction could be detected (Figure 5).

A co-ordinative expression of c-kit and kitLG was seen in the intact mucosa: the same but of lesser extent was present in the laryngeal tumors, whereas in the pharyngeal tumors the decrease was more pronounced (Figure 6).

DISCUSSION

Overexpression of growth factor receptors, including IGF, EGF, TGF-alpha, kitLG and PDGF receptors, has been associated with poor prognosis in many cancers.[12,17,21] Therefore, a number of receptor tyrosine kinases (RTKs) are already targets for novel designed drugs, which include tyrosine kinase inhibitors and monoclonal antibodies.[10] Despite the fact that c-kit and PDGF-R turned out to be effective targets in a number of cancers, the experimental results in HNSCC have not confirmed their importance yet. The expression and function of c-kit in HNSCC is a quite controversial subject.[16] In this work changes in the co-expression patterns of kitLG and its receptor in head and neck cancers were studied. Correlation of gene expression with tumor characteristics and clinical course of the disease has only been demonstrated in nasopharyngeal cancers in relevant publications.[18] Co-expression as such was found to be associated with poorer prognosis.[2] It was demonstrated in Merkel cell carcinoma, melanoma, glioma and breast cancer that an autocrine growth signaling pathway develops along the kitLG–c-kit axis in the tumor cells.[9,13,14,20]

In GIST the c-kit is diagnostic and serves as a target for molecular therapy.[1]

Our results suggest that the main source of kitLG and c-kit production in the head and neck cancer is the mast cell present in the microenvironment of the tumor. It cannot be excluded that the epithelial cells also express the ligand and/or its receptor but the resolving power of the immunohistochemical method failed to reach the threshold value of detection.[7]

If the tumor cells secrete the kitLG ligand then they are able to exert their paracrine effect on other target cells, as well. Through the specific receptor expression they may set the autocrine pathway into motion.[19]

It is an established observation that kitLG produced by tumor cell plays some role in attracting the mast cells to the tumor periphery.[11,4]

Mast cells may occur in many tissue types.[6] They are constituents of the normal connective tissue stroma. However, they may appear in several physiological and pathological processes, for instance in wound healing. In a certain sense the tumor itself can be regarded as a non-healing wound.

The presence of mast cells at the tumor periphery was reported in diverse human neoplasms.[3,5] Mast cells are parts of the microenvironment of the tumor. They are typically found in the connective tissue stroma, localized mainly to the vicinity of capillaries. They do not infiltrate the tumor. Interestingly, aggressive human cancers are usually associated with a dramatic host response by various inflammatory cells, especially mast cells at the tumor periphery.[15]

Once a tumor forms, the transcription of angiogenic factors increases. As neoplastic progression proceeds, angiogenic growth factor gene expression is up-regulated in the cancer cells, pushing progression to the second cancer phase, wherein the tumor cells control their angiogenic phenotype directly instead of depending on the manipulation of inflammatory cells to indirectly affect neovascularization. Thus, overtly malignant tissues that constitutively express high levels of angiogenic factors and ECM-degrading proteinases may no longer require the assistance of mast cell-derived factors.

To undertake this role it is enough if the much bigger individual tumor cell produces the given angiogenic factor in a relatively smaller amount than the mast cell.

Thus, despite the process described the tumor cells may happen to be negative in contrast to the mast cells being far less in number.

Based on our gene expression and immunohistochemical examinations it is presumed

that for the co-expression of local kitLG and c-kit, the mast cells are responsible; in the intact tissue almost exclusively, and in the squamous cell carcinomas that express the two factors at low level, primarily. Hence it follows that the decreased expression in the tumor specimens compared to the intact mucosa and the further fall in the metastases can be interpreted as if the dedifferentiated tumor would emancipate itself from the adjacent mast cells after the completion of initiation, moreover as if it would overgrow and come to a larger proportion in the tumor specimen than in the mucosa. It is supported by the fact, that in our specimen distribution the hypopharyngeal tumors had higher grades.

Provided that our hypothesis is correct, the expression of kitLG and c-kit does not increase in the mast cells at the tumor periphery. It is presumed that the mast cells do not activate through the direct raise of kitLG and c-kit the growth factors needed for the support of neoangiogenesis.

Perhaps methods with higher resolving power would contribute to the better understanding of this problem. By means of them the normal mast cells could be compared with those co-operating with premalignant cells and those present in the vicinity of an advanced tumor.

CONCLUSION

Mast cell migration to the tumor site and their subsequent activation may be a prerequisite for their promoting effect on tumors. In this respect, stem cell factor is possibly involved, because it triggers off the c-kit signaling pathway for the differentiation, migration, maturation, and survival of mast cells.[7] In the present study, we investigated the relation of mast cells and kitLG/c-kit in tumor progression. The remodeling of the tumor microenvironment can actually be initiated by tumor cell-released cytokines/chemokines.

This process is subject to the actual interrelationship between mast cells and tumor cells, and mast cells and other immune cells, respectively. Therefore, the kitLG/c-kit pathway is very important not only for the remodeling of tumor microenvironment but also for target tumor therapy.

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Persistent organ failure - factor of fatal outcome in severe acute pancreatitis

Popa D, Copotoiu C, Chindriș V, Russu C, Panțîru A, Pocreață D

Background: Severe acute pancreatitis (SAP) is a disease well known for its wide spectrum of severity, complication and outcome. Many patients develop transient or persistent organ failure (OF) and they can recover completely or develop fatal complications. It has been suggested that persistent OF in first week of SAP is associated with more complications and high mortality. Method: The aim of the study is to determine mortality rates and complications in patients with transient (<48hours) or persistent OF (>48hours) in a group of 279 patients with SAP treated in 1st Surgery Clinic from Tg.Mures between 1995-2009.Results: Early OF was present in 143 patients. After transient OF (n=76) outcome was good (mortality=10,52%) and local complications was recorded in 25% of cases. Persistent OF(n=67) was associated with high mortality(49,25%) and 56,71% local complication. Conclusion: Duration of organ failure during the first week of predicted severe acute pancreatitis is strongly associated with the risk of death or local complications. Resolution of organ failure within 48 hours suggests a good prognosis.

Key words: severe acute pancreatitis, organ failure, mortality

Severe acute pancreatitis as a common acute abdomen is characterized by complicated causes, lots of morbidities and high mortality. Despite considerable improvements in understanding of its pathophysiology and modern therapy of this patients, morbidity and mortality remains high (mortality range between 15-40%).[1] Approximately 30-50% of the deaths in SAP occur during the first week, as a result of progressive organ failure(multiple organ dysfunction syndrome-MODS).[7,10] In contrast late deaths (more than 7 days after onset of SAP) are often associated with major complications, such infected necrosis, that are triggers for sepsis and MODS.[3,6,11] Clinical experience show that many patients with early OF, one of the Atlanta criteria for severity, respond rapidly to treatment and have an uncomplicated evolution. OF is a dynamic process and the progression of it in first week of SAP is associated with a mortality greater than 50%.[7]

MATERIAL AND METHOD

The present study reviews a database of 279 cases with SAP from 1159 patients with acute pancreatitis treated between 1995-2009 in 1st Surgery Clinic from Tg.Mures. These patients, aged over 17 and less than 88 years, have an APACHE II score > 6 or Ranson's score >3 in the first 24 hour and have a sex ratio male :female 1,6:1. Organ failure were present in 143 patients (62,74%) and appreciated using Atlanta criteria (cardiac failure if systolic blood pressure was <95mmHg or need inotropic support, central nervous system failure if Glasgow coma scale was <13, coagulopathy if platelet count was < 90.000/ml, renal failure if plasma creatinine was > 3 mg%, oligo-anuria or need dialysis, respiratory failure if the SaO_2 was <90% or need respiratory support, hepatic failure if serum bilirubin was greater than 3 mg/ml, protombine time >15s.[2] Transient OF (TOF) was defined as organ failure present less than three days and persistent OF (POF) when present three or more days after admission.

RESULTS

The database included 279 patients with SAP of whom 53 died (total mortality 16,63%).

Table I. APACHE/ Ranson score in first 48 hours and mortality

APACHE score	Ranson score	survived	deaths	mortality
6	3	22	0	0
7	4-5	18	2	11,71%
8	6	96	12	12,37%
>8	>6	143	39	27,27%

Table I shows a strong associations of a fatal outcome with an APACHE II score >8 or Ranson score greater than 6 (mortality 27,27%). Of these 53 deaths 20 occurred within the first week; all these patients had OF from the day of admission until the day of death. The rest of 33 deaths occurred in more than one week (median 18,9 days; range 8-98 days), 7 late deaths (more

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than 6 weeks) were found not to be due to SAP, and the rest of 26 deaths were related to complications of SAP. The relations between the presence and persistence of OF within first week and subsequent deaths is show in table II.

Table II. Type of organ failure (OF) and mortality

	survived	deaths	mortality
No organ failure	114	5	4,38%
Transient OF	76	9	11,84%
Persistent OF	67	33	49,25%
Late OF(7 days)	22	6	27,27%

Only 59,13% of patients already had OF at the admission and POF (24,01%) was responsive for 33 of deaths, instead TOF (27,24%) was the cause of only 9 deaths. Of the patients who died 5 had no OF, 19 had a single OF and 29 had two or more OF (Table III).

Table III. Type of organ failure (OF) in died patients

Organ failure	deaths
No OF	5
Single OF	Respiratory failure 10
	Renal failure 9
Multiple OF	Renal & pulmonary 7
	Pulmonary & cardiac 4
	MSOF 18

Of 143 patients with OF during first 7 days, 69 developed local complication and only 22 in the subgroup of 136 patients (Table IV).

Table IV. Organ failure and complications in SAP (pancreatic abscess, fistula, pseudocyst)

Organ failure (OF)	no	Local complications	%
No OF	136	22	17,17
Transient OF	76	19	25%
Persistent OF	67	38	56,71%

DISCUSSIONS AND CONCLUSIONS

We can conclude that is a strong relation between the duration of OF in SAP, mortality and local complications. The observation that approximately half of the patients with a raised APACHE II or Ranson's score had organ failure in the first days after admission was associated with fatal outcome suggested that the progress of OF despite aggressive treatment could be a useful marker for severity.[7] Butter et al. predict the severity of acute pancreatitis using a cut t off APACHE II score >6 and found that POF was associated with mortality greater than 50%(3). Our study shows that the group of patient with POF was much greater (49,25%) than the mortality in patients with TOF (11,84%) and an APACHE II score greater than 8 is followed by death in 27,27% of cases. The first week represents a pike in the development of systemic inflammatory response which characterized SAP and management of this phase is critical. The POF is associate with late deaths and the presence of late complications.[4] POF in first week appear to be associate also with presence of extensive pancreatic necrosis as a trigger for organ failure or a consequence of it. The relation between organ failure and necrosis is not well known but some studies found a greater mortality in infected necrosis associated with multiple OF.[8,9] Transient organ failure was associated with good outcome and most of patients had a single OF[8], usually pulmonary or renal. Almost certainly the POF leads to local and systemic hypoxia and perhaps hypoperfusia that could be the factors for extensions of necrosis and occurrence of infection. Aggressive circulatory support with intravenous fluids, oxygen therapy might lead to improve the microcirlatory status and reduce the risk of necrosis and death.[5] In conclusion the patients with SAP and dynamic and persistent organ failure (more than three days) have a greater risk to develop locals complications and for fatal outcome than those with transient organ failure.

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Vaginal birth after caesarian operation

Shaer M. A.

Many clinicians remain uncertain about a trial of labor in women who have had a cesarean delivery, mostly because of concern about uterine rupture. This retrospective analysis, designed to determine the rate of vaginal birth after cesarean delivery (VBAC), comprised 18102 deliveries occurring at our obstetrical hospital during a 12-year period from 1993 to 2004. It is noticeable that caesarian interventions rate was growing each year and the number of caesarian operation on scarred uterus grew along. In some centers for birth a majority of women with previous operative delivery now are delivering vaginally. There is a definite risk of uterine rupture with potentially serious consequences, making informed decision of the utmost importance. Any woman with scarred uterus who opts to attempt vaginal delivery must be hospitalized in obstetric service, intensively monitored and must have immediate access to a surgical facility.
Key words: caesarian operation, scarred uterus, birth

The birth process coming after caesarian intervention or on any kind of scarred uterus is a debated situation by modern obstetrics. Is important to complete an observation sheet including details about female, her general and obstetrical history and observations. The purpose of this study was to identify the results after vaginal birth on scarred uterus (VBAC) and to report maternal and neonatal outcomes. The associated elapsed time window for delivery of an uncompromised neonate was also investigated. A retrospective study was performed. The aim of the study was to show experience about vaginal birth on scarred uterus (VBAC) and the risk of method. But caesarian intervention in the same situation is not risk free.[6] So remain of clinician obligation to decide in each case the better birth intervention.

MATERIAL AND METHOD

There were 18102 births in 12 years, 16410 vaginal births and the other 1791 were caesarian births, form this 77 vaginal birth on scarred uterus and 405 caesarian on scarred uterus. All female with scar uterus were completed an observation sheet including the age, social situation, personal and familial history, physiologic history with dates about first menstruation and interval, duration of menstruation and about other births, age of pregnancy, mentioned details about caesarian operation or any other kind of uterine scare. If their was any kind of complication in obstetrical history, complication after caesarian section, fever, infections and fetus condition and complications were detailed. Were also studied some parturients characteristics like pelvis status, cervix situation and fetus presentation and fetus weight at birth.

Obstetrics and Gynecology II Clinic, Târgu Mureş

All clinic data were noted retrospective from observation sheets, operation and hospitalization registers.

RESULTS AND DISCUSSIONS

From 18102 birth registered in the clinic in 12 years, 16410 were finished by vaginal way. 77 VBAC from total of 16410 vaginal birth was represented by percentage in the chart (Figure 1).

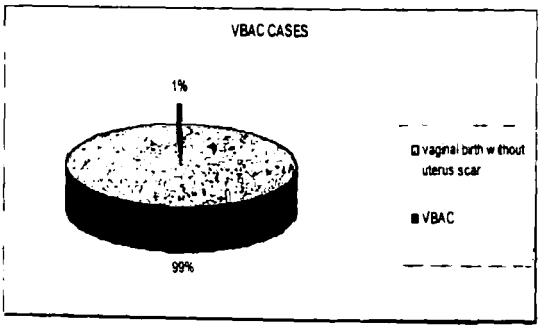

Figure 1 - VBAC Cases

fetus in cranial presentation, 1 pelvic and 1 face presentation. No case was found in transverse presentation (Figure 2).

As was showed, 98% of fetus were in normal cranial presentation.

One of the most important to study was the pregnant pelvis checked, the data had been mentioned in observation sheet. Diagnosis of pelvis situation and decision of the necessity of caesarian operation or for a vaginal birth was made after external pelvimetry,

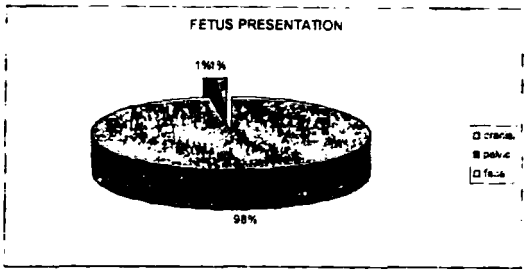

Figure 2 - Fetus Presentation

Rhoumbus of Michaelis evaluation, internal pelvimetry made by emergence obstetrician. All results had been noted on observation sheet.

From all 77 pregnant with VBAC, 72 (94%) had eutocic pelvis and 5 (6%) narrow pelvis. No limited or android pelvis were found (Figure 3).

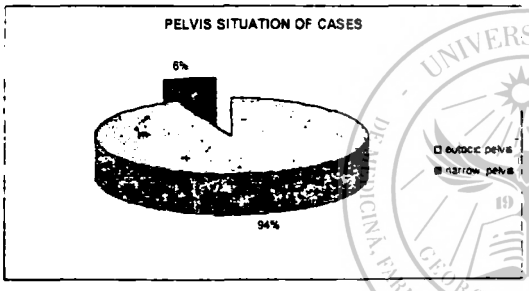

Figure 3 - Pelvis Situation Cases

An other element analysis from observation sheet was the situation of cervical dilatation of parturients in the moment of hospitalization. From 77 VBAC pregnant were noted 35 cases (45%) with cervical dilatation of at least 4 cm and 42 (55%) with intact membranes and no dilatation.

An other criterion was fetus weight at the birth and were found 1 newborn under 1000 g, 9 with weight between 1001 and 2500 g, 25 between 2501 and 3100 g, 40 fetus with 3101- 4000 g and 2 after 4000 g. (Figure 4).

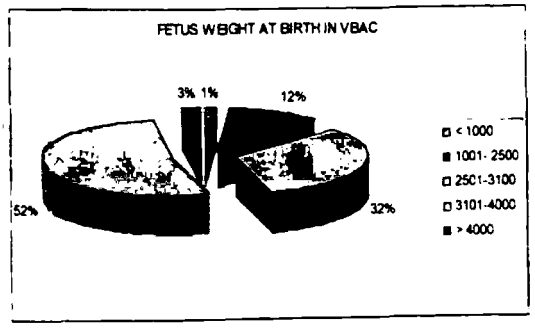

Figure 4 - Fetus Weight at Birth

To develop recommendations for the preferred delivery method for a pregnant woman with VBAC the results of this study show the following observations. 87% of fetus were registered having more then 3100 g, that suggest the scarred uterus did not determined a small and abnormal weight and the labor could be performed vaginal for fetus having even more than 4000 g. In literature, based on reported data indicating that about 70 percent of those who attempt VBAC will deliver vaginally, the estimated rate of uterine rupture was 0.34 percent.[2] In that study were not significant complications and 1 fetus not survived in the first 6 days, 2 of them were Apgar under 3.

When planning a VBAC it is important to determine if the previous low vertical scar has not stretched to the body of the uterus in the current pregnancy. Sometimes a woman may have a "T" or "J" shaped scar on the uterus or one that resembles an inverted "T". These scars are very rare. It is estimated that between 4 and 9% of "T" shaped uterine scars are at risk for rupture.[5]

CONCLUSIONS

VBAC represented 0,42% of total birth in our clinic. Neonatal and maternal complications in VBAC were no significant because of prompt intervention.

An important factor to decide between urgent caesarian operation or VBAC in the situation of scarred uterus is membrane status at hospitalization moment.

No mothers died, and was no significant morbidity. Measuring the thickness of the uterine scar from a cesarean delivery[3], external pelvimetry, Rhoumbus of Michaelis evaluation, internal pelvimetry can help physicians predict which women would be at low risk for uterine rupture or scar reopened and may safely try to have a vaginal delivery,

For women who are planning to have more children after their VBAC, having their VBAC in a birth centre were successful VBAC rate is the same as in hospital is an important decision.[4] That will result in better outcomes in terms of maternal and infant mortality and morbidity due to the lower repeat caesarean section rate.

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Vascular endothelial growth factor increases vascular permeability in acute respiratory distress syndrome

Raluca Solomon[1], L. Azamfirei[2], Simona Gurzu[3], Ruxandra Copotoiu[2], Sanda Copotoiu[2], I Jung[3], J. Szederjesi[2], Judit Kovacs[2]

Objective: investigation of the VEGF expression in the lung tissue of ARDS patients and also the VEGF plasmatic levels of these patients.
Material and method: there were examined immunohistochemical lung specimens from 10 ARDS patients which were compared to a control group. The VEGF seric level was determined using ELISA method, at the same time with the tissue sampling from ARDS patients and during the hospital stay in case of the control group of non ARDS patients.
Results: patients who died because of ARDS had VEGF pulmonary expression significantly decreased comparing to non-ARDS patients ($p<0,001$). The seric VEGF levels of ARDS patients were raised comparing to non-ARDS patients ($p<0,001$).
Conclusions: A decreased alveolar type II cells, was noticed in ARDS evolution, therefore reducing the VEGF production in the alveolar space and also contributing to the decrease in lung perfusion, but also to the consecutive increase of VEGF plasmatic level.
Key words: ARDS, Vascular endothelial growth factor

Acute respiratory distress syndrome (ARDS), the most severe form of acute lung injury (ALI), remains a devastating condition characterised by difuse injury of the alveolo-capillary wall and alveolar and interstitial oedema consecutive to increased pulmonary vascular permeability. It concerns 1,5-8,3 cases in every 100 000 patients, still having a signifiant mortality of 30-50% despite improvements in the management of sepsis and lung protective ventilation strategy.[2] There is a heterogeneous group of conditions, both direct or indirect insults, such as sepsis, trauma, aspiration, massive blood transfusion or burns which predispose to ARDS. The pathogenetic basis of ARDS and factors governing susceptibility are incompletely understood. Markers of both epithelial and endothelial injury have been correlated with outcome[8], wheras the severity of ARDS depends significantly on the balance between alveolar epithelial and/or vascular endothelial injuries and their repair mechanisms.[13]

Vascular endothelial cell growth factor (VEGF) was identified by its properties to increase permeability and act as a cellular growth factor, hence its potential for a key role in the pathogenesis of ALI/ARDS. The VEGF family has several members, and each acts through specific receptors. The human VEGF gene family includes VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placenta growth factor (PlGF), all with multiple and diverse biological functions. The genes for VEGF family members also rely on alternative exon splicing to confer various isoforms for biological and functional specificity.[9] The most studied molecule of the VEGF family is VEGF-A. Its double effect, both permogen and mitogen[3] induces vascular endothelial cell proliferation and promotes survival by induction of anti-apoptotic proteins bcl-2 and A1.[5] VEGF increases microvascular permeability, but also it also exerts bioactivity outside the vascular endothelium: macrophages, type II pneumocytes, and monocytes for which it may be chemotactic.[4] The biological activity of VEGF is dependent on interaction with specific receptors (VEGF-R1, 2, and 3), which are expressed not only by endothelial cells, but also by activated macrophages and alveolar type II epithelial cells.[1] VEGF acts also on a family of coreceptors, the neuropilins.[10]

The objective of this study was to investigate the VEGF expression in the lung tissue of ARDS patients and also the VEGF plasmatic levels of these patients compared to a control group.

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MATERIAL AND METHOD

We realized a prospective study including 10 patients diagnosed with ARDS. These patients were evaluated daily during their stay in the ICU and those included in this study accomplished the ARDS diagnosis criteria as recommended by the international American-European consensus conference. The parameters needed to assess the SOFA score were determined daily and were refered to the score obtained on the fist day of setting the ARDS diagnostic.

Lung specimens from ARDS patients were obtained by bronchoscopy or by autopsy in case of the deceased patients. The controls were 10 patients deceased from other causes than ARDS from whom necroptic pulmonary tissue samples were obtained and processed as for the ARDS patients.

All lung samples were standard stained using Haematoxylin Eosine and were examined in order to assess the histopathological diagnosis of ARDS: presence of hyaline membranes, type II pneumocyte proliferation associated with myofibroblast proliferation.

The determination of VEGF expression in the pulmonary tissue was performed using specific monoclonal antibodies VEGF, clones VG1 and JH 121 produced by LabVision firm. A charge-coupled video camera coupled with an optical microscope, was used to view the sections and to digitize images to a PC host computer using a program of assisted quantification. At least 10 fields per section were analysed and the results were given as the ratio (%) of stained surface area over the total field surface area.

The VEGF seric level was determined using the ELISA method, at the same time with the tissue sampling of the ARDS patients and during the hospital stay in case of the control group of non ARDS patients. Plasma probes were aquired for VEGF measurement using the Human VEGF ELISA kit with crossed reactivity for VEGF 165, VEGF 121 and PGDF. (BioLife Group).

The statistical analysis of data was made using media and standard deviation through soft SPSS, version 17.0.

RESULTS

The ARDS etiology of the studied patients was mainly extrapulmonary (7 cases out of 10) (Figure 1) and the demografical and clinical data of the ARDS patients appear in Table I.

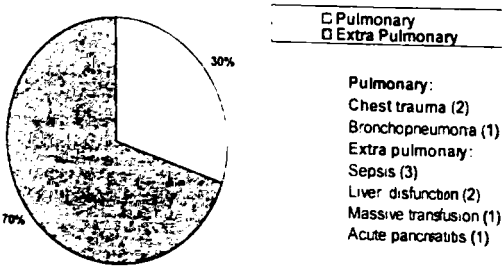

Figure1. Causes of ARDS

Table I. Demographic and clinical data of ARDS patients

Parameters	Value
Age	52 (31-71)
Male/Female	6/4
PaO2/FiO2 (average, extremes)	128 (61-191)
Period of mechanical ventilation (average days, extremes)	11,37 (4 – 26)
ICU stay (average days, extremes)	18,37 (4-39)
SOFA score (the ARDS diagnostic day) (average, extreme values)	6 (4-9)
Mortality	40% (4/10)

The mortality of ARDS patients was of 40 % (4 out of 10 patients). PaO2/ FiO2 ratio was lower than 200 for all the ARDS patients according to one of diagnostic criteria, but this ratio will be subsequent modified for the survivors (assessed in the day of extubation) comparing to the non-survivors (assessed in the day of death), with a mean of 282 for survivors versus 128 for the non-survivors ($p<0.05$) (Figure 2).

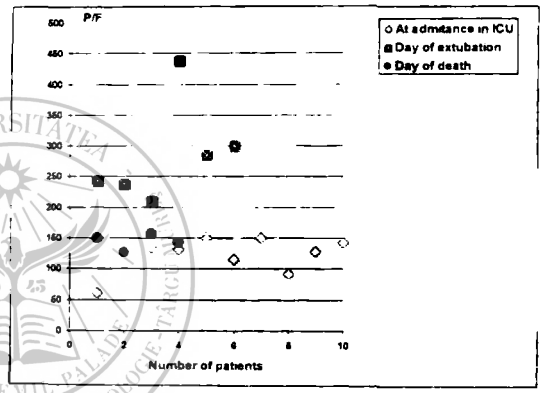

Figure 2. Dynamic of PaO2/FiO2 ratio in ARDS patients

The mean period of ICU stay was 18 days (with a maximum of 39 days) and the mean ventilation period was 11 days (with a maximum of 26 days). The mean SOFA score aquired in the first day of ICU stay was 6, with similar values for survivors and for the non survivors.

Patients who died because of ARDS had a VEGF pulmonary expression significantly decreased compared to non-ARDS patients: 8,5 (4,1-9,9) versus 28,7 (9,5-48,6) ($p<0,001$) (Figure 3).

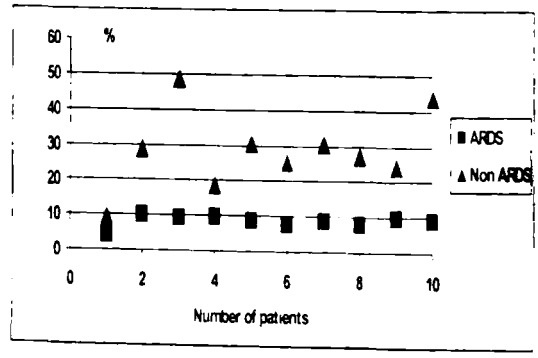

Figure 3. Distribution of VEGF expression in ARDS vs non ARDS patients

On the other hand, the seric VEGF levels of ARDS patients were raised (230 pg/ml) compared to non-ARDS patients (131 pg/ml) (p<0,001) (Figure 4).

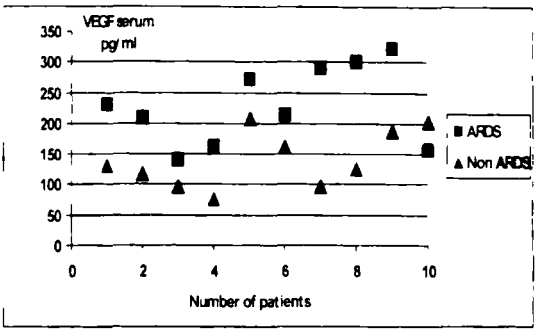

Figure 4. Distribution of serum VEGF level in ARDS vs non ARDS patients

Alveolar macrophages were immunopositive in both groups.

No significant statistical differences were noted between the two groups with regard to age, gender, period of ARDS conditioned, number of ICU days.

DISCUSSION

In the normal lung tissue, VEGF is highly compartmentalised, with levels within the alveolar space 500 times than over the plasma figures[8], despite VEGF production being closely associated with hipoxia response element. In vitro studies have demonstrated an abundance of VEGF especially in the alveolar epithelium,(including the A549 cell line and primary human cultured type II pneumocytes) which suggests that the alveolar epithelium is the predominant source.[1,12] The expression of VEGF in ARDS varies, depending of epithelial and endothelial damage. In the early stages of ARDS plasma VEGF levels rise and intrapulmonary levels fall, with normalisation of both during recovery.[11] A possible explication of this variation is that the alveolar injury is the primary modulator of the alveolar level of VEGF in ARDS. This variation is probably the consequence of VEGF degradation by proteases released from infiltrated neutrophils and other inflammatory cells in the alveolar space.

VEGF release takes place in 3 phases paralleling the development of ARDS. The initial injury of the lung and the proinflammatory cytokines stimulate the production and the release of VEGF from type II pneumocytes, alveolar macrophages and marginal neutrophils.

Subsequently, the endothelial-alveolar barrier is exposed to high concentration of VEGF, which alters the adherence jonction complexes (AJC) leading to microvascular injury and interstitial oedema. As the ARDS lesions develop, there is a distruction of type I and II alveolar epithelium, with a release of protease from neutrophils which leads to the decrease of VEGF concentration in the alveolar compartment. This pulmonary compartment deprivation of VEGF besides the release of VEGF from other organs increases the VEGF seric concentration, as ARDS represents only the pulmonary manifestation of a widespread endothelial injury in multiple organs. During the recovery period of ALI (when this appears!) type I and II alveolar epithelial cells begin to reapear, the VEGF production rises, wich may participate in the angiogenesis, an important component of lung repair.

The over-expression of pulmonary VEGF, leads to increased vascular pulmonary permeability and consequently pulmonary oedema.[6] Nevertheless, the VEGF expression in human alveolar epithelial cells also facilitates neovascularization, contributing to endothelial injury repair.[9]

Low VEGF pulmonary levels were associated with the severity of ARDS, whereas high VEGF levels were associated with the recovery from ARDS, signalling the role of VEGF in the pulmonary injury repairing process.[11]

CONCLUSIONS

A decreased alveolar type II cells, induced by apoptosis was noticed in ARDS evolution, therefore reducing the VEGF production in the alveolar space and also contributing to the decrease in lung perfusion, but also to the consecutive increase of VEGF plasmatic level.

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Family affected by astrocytoma. Case report

Antonela Ciobanu, Ingrith Miron, I. Tansanu

We report a rare case of familial clustering of astrocytomas (tumor of central nervous system) with 2 brothers from 5 affected, 2 years one from another.
The boy R.V.I. age 5 and 3 months was diagnosed with IV-th ventricle grade I astrocytoma; after total resection of the tumor he underwent chemotherapy for one year but 3 years later he developed local relapse. The second surgery was followed by chemotherapy.
His sister, R.G., diagnosed at 13 years and 10 months of age with brain stem anaplazic astrocytoma grade III died 5 months afterwards. She underwent surgical biopsy followed by chemotherapy.
It was unable for us to prove the implication of several genetic or environmental factors described in literature but their influence is not neglectable due to the same histopathological type of tumor.
Key words: familial clustering, astrocytoma, diagnose, evolution

This material present the case of a family where 2 of the 5 siblings (a boy and a girl) harbored central nervous system (CNS) tumor – astrocytomas.

The two cases are R.V.I., a 5 year and 3 months – old boy, diagnosed in 2005 with pilocytic astrocytoma of the IV-th ventricle and his sister, R.G. aged 13 years and 10 months, with anaplastic brainstem astrocytoma in 2007.

The medical files were analised for this report.

The first case was a boy (the 5-th born of this family) who was hospitalized with a 2-week history of headache, nausea, nistagmus and visual impairment. CT scan showed the presence of a IV-th ventricle tumor of 37/31/29 mm with obstructive hydrocephalus and diffuse cerebral edema (Figure 1,2).

He underwent total mass resection followed by histopathological and immunohistochemical analysis showing a grade I pilocytic astrocytoma with low expresion of Ki-67 antibody in tumoral cells (demonstrating their low proliferation rate).

Postoperatively he received Temozolomide 150/ mg/sqm/day, 5 days, repeated each 28 days, for one year, associated with symptomatic therapy. During the therapy we noticed emetic syndrome and hematological side effects (neutropenia – 5 events and thrombocytopenia – 8 events).

One year after he presented with high intracranial pressure signs; CT scan showed obstructive hydrocephalus without tumor relapse and he was treated with cerebrospinal (CSF) diversion, a ventriculoperitoneal shunt.

Three years after the diagnose, in 2008, he developped local relapse and he underwent a third surgery followed by a second chemotherapy. It was the same histopathological diagnose.

His sister, R.G., aged 13 year and 10 months (the first born of the family) presented in 2007 with visual loss, right facial palsy, vomiting and headaches.

An MR image of the brain revealed a large enhancing mass in the brainstem with cerebellar extension and obstructive hydrocephalus (Figure 3, 4).

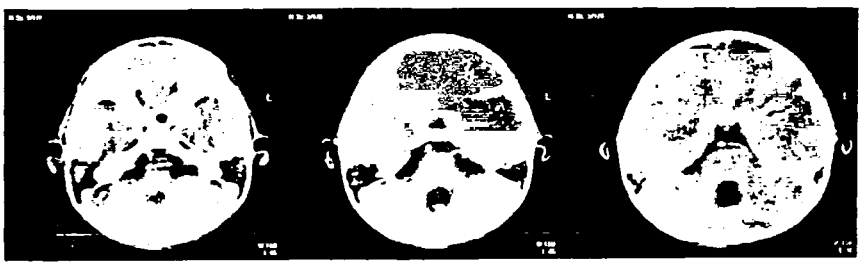

Figure 1. Preoperative, precontrast, serial CT scan axial images in patient R. V. I.

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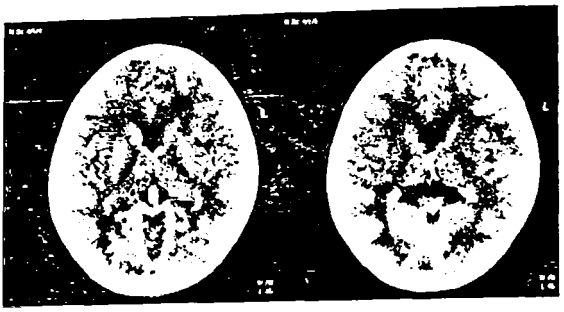

Figure 2. Preoperative, postcontrast, serial CT scan axial images of the same patient.

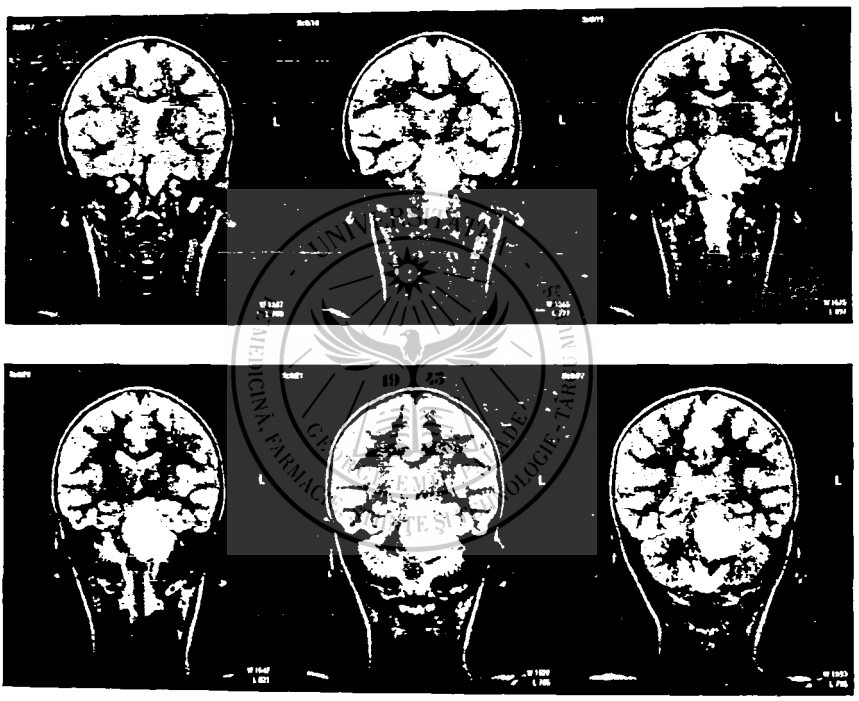

Figure 3 Preoperative,coronal, postcontrast serial MR images in patient R. G.

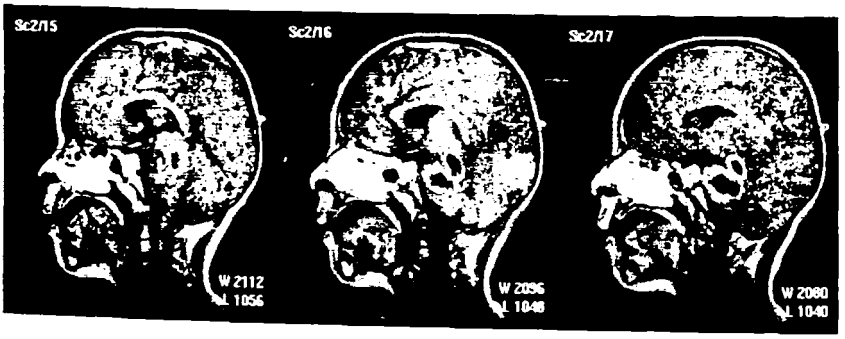

Figure 4. Preoperative, sagital, precontrast serial MR images of the same patient

This particular location only allowed a surgical biopsy with ventriculoperitoneal shunt and histological examination diagnosed anaplastic astrocytoma grade III.

She received Temozolomide 150/mg/sqm/day, 5 days, every 28 days, associated with adjunct therapy. She developped side effects and complications: urinary infection, gastro - intestinal toxicity (mucositis, constipation) and secondary amenorrhea.

Despite this treatment the evolution was unfavourable with progressive clinical and neurological deterioration; she died 5 months after.

DISCUSSION

Astrocytomas are tumors derived from an immortalized astrocyte and represent the most frequent types of CNS tumors in childhood with different grades of anaplasia according to World Health Organisation: pilocytic astrocytoma corresponds to grade I, low - grade diffuse astrocytoma corresponds to grade II, anaplastic astrocytoma corresponds to grade III and glioblastoma multiforme corresponds to grade IV.[6]

The etiology accepts various risk factors and exposures, from radiations, electromagnetic fields, viral infections, certain drugs and chemicals[3,4,7,10] to genetic factors.[5,8]

Familial clustering of astrocytomas have been well documented in the literature since the end of XIX-th century linked with inherited neoplastic syndromes such as Turcot syndrome, neurofibromatosis type 1 and Li - Fraumeni syndrome.[2,6]

The symptoms depends foremost on the site and extend of tumor: headaches, nausea, vomiting (signs of increased intracranial pressure), cognitive impairment, visual disturbances, motor impairment, seizures and ataxia.[6] A detailed neurological examination is followed by imaging studies such as CT scan and MRI (with and without contrast) to establish tumor location and extension.

Treatment of astrocytomas includes maximal surgical resection with/without cerebrospinal fluid diversion and histological evaluation followed by chemotherapy and/or radiotherapy.[1,6] The immunohistochemical analysis of Ki 67 antibody may reveal the proliferation rate of tumoral cells.[9] The follow - up surveillance and free-event survival, overall-survival and relapses are linked with individual factors and histopathological findings.

The siblings we report had both astrocytomas but sex, age, location, malignancy and evolution were completelly different.

It was unable for us to prove the implication of several genetic (inherited neoplastic syndromes such as Turcot syndrome, neurofibromatosis type 1, Li - Fraumeni syndrome) or environmental factors described in literature (viral infections, radiations, electromagnetic fields, drugs and chemicals). There was no relevance in the family genealogic tree. Two-third of low-grade astrocytomas has p53 mutation so the action of some undetermined genetic factors should be considered.

CONCLUSIONS

The existence of familial CNS tumors was first described in the literature in 1896; several cases have been described since then. Furthermore, the literature includes descriptions of families were the patients were siblings.

The pattern of brain tumor occurrence in families has been attributed to environmental, multifactorial causes or to autosomal recessive inheritance.

The clinical characteristics of the boy: small age, total tumor resection, grade I of anaplasia and low expression of Ki 67 antibody in tumoral cells represent favourable prognostic factors; despite this he relapsed and needed another 2 surgery and chemotherapy. The girl had only unfavourable prognostic factors: older age, no gross resection of the tumor, high grade of anaplasia.

Such cases need multimodal management and recall psychological suport and genetic counseling for the whole family.

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Prader - Willi Syndrome – Case Presentation

Simona Coşoveanu[1], D. Bulucea[2]

The paper presents the case of a child, female, aged 3 years and 2 months, with obesity, obvious muscular hypotony since an infant, neuropsychic retard, hypogonadism, a clinical aspect which leads to the Prader-Willi syndrome. The clinical suspicion for the diagnosis was followed by a cytogenetic examination for identifying a deletion or some chromosomal rearrangements which imply the 15q11.2-q13 region. The result was confirmed by the DNA study – molecular analysis (Institute of Clinical Genetics within the Olga Hospital in Stuttgart): there is a deletion of the paternal band for the methylation test, thus confirming the clinical diagnosis of Prader-Willi Syndrome.
In order to clarify the exact cause (deletion, maternal UPD, imprinting faults) and to define the recurrence risk in additional children, we suggest a micro-satellite analysis to include DNA samples from both parents. The treatment aims at limiting polyphagy, monitoring weight, imposing a diet, physical exercises and an educational program which should improve the behavioral status.
***Key words**: obesity, Prader-Willi syndrome, diagnosis, children*

Prader-Willi Syndrome (PWS) is characterized by obesity, marked muscular hypotony since an infant, neuropsychic retard and hypogonadism with an added nanism. PWS is caused by the absence of the paternal allele gene expression in the 15q11.2-q13 region. The incidence is 1 to 10000-15000 (State & Dykens, 2000).

Clinically, the main symptoms are emphasized in 2 phases. During infancy and little child period, they are obvious: congenital muscular hypotony (poorly marked movements), respiratory and feeding difficulties, nanism, obesity (progressive), psychic retard and hypogenitalism in both sexes. Complementary symptoms: narrow forehead (sometimes large and bulged), big, almond-shaped eyes, strabism, triangular, half-opened mouth, hypoplasia of dental enamel, early tooth cavities, bone anomalies (extremely short hands and legs – acromicria, acromelia which involve the forearms and shanks, a twisted varus-echin leg, cifoscoliosis), umbilical hernia. In the second phase, the obesity worsens, an abnormal TTGO, diabetes mellitus occurs, but without a tendency to ketosis.[3]

The DNA-based methylation analysis and a fluorescent in situ hybridization (FISH) confirms the diagnosis of PWS (Richer, Shevell, & Miller, 2001).

Case presentation

The paper presents the case of the child D-V. E. L., female, aged 3 years and 2 months, from an urban area, admitted (A.F. 27552/ 2008), in the 2nd Pediatric Clinic of the Emergency County Hospital in Craiova, for a diagnosis in obesity and the presence of a respiratory functional syndrome.

The anamnesis reveals that the patient is the second child of a young affirmatively healthy couple, the father and two aunts (on behalf of the father) presenting obesity level I/II. Spontaneous, natural, full term-birth, weight at birth= 2600g, Apgar score and immediate postnatal evolution can not be mentioned by the mother. She was gavage-fed for 3-4 days, and breast-fed for 3 weeks (a difficult feeding), then artificially fed with various milk formulas, incorrectly diversified since the age of 4 weeks; since the age of 5 months he ate adults' food, an excessive alimentation. He followed the rickets prevention program until the age of 1 year; current vaccines according to WHO scheme.

Belated physical and psychomotor development on age periods: she held her head at 4 months, she sat at the age of 8 months, she walked held by axils at the age of 1,5 year, she babbled at the age of 3-4 months, monosyllabic sounds at the age of 10 months, she uttered her first word at the age of 1 year, at 2 years a motor development which corresponds to the age of 10-11 months, she walked by herself at the age of 2,5 years; now, she is speaking with difficulty, 15 no connected words in a sentence, she is playing.

Personal pathologic antecedents: at the age of 4 months – hypotone diplegia (Obregia Hospital, Bucharest) for which she followed a drug treatment and medical recovery; cranial CT (Marie-Curie Hospital, Bucharest): ventricular system on the median line, with discrete asymmetry at the level of the frontal horns, white substance differentiation, grey substance present. Prepontine cistern, enlarged subarachnoid areas. Left temporal arachnoid cyst. Bilateral fronto-parietal micro-lacunar images. At 11 months reductible varus equin leg (discrete tricipital pressure); repeated

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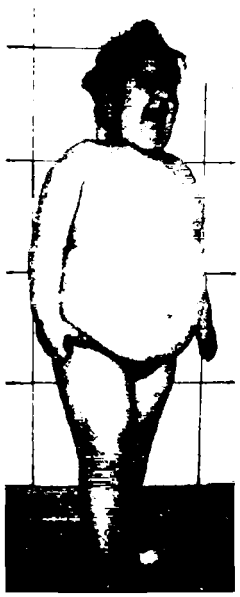
intercurrent respiratory diseases which led to hospitalization.

The anamnesis reveals that the child's state worsened all of a sudden, presenting abundant perspiration, oral-type respiration, dyspnea caused by effort, intercostal draught, perinasal cyanosis.

Objective examination at admission: generally influenced state, no fever, wet and marbled teguments, cold extremities, venous circulation visible on anterior thorax and abdomen, perioronasal cyanosis, inspiratory dyspnea, intercostal, subcostal draught, RF=30/min., VA=110/min., BP=90/50 mmHg, facies in "full moon" shape, ginecomasty, hairiness at the level of the posterior thorax, of the upper and lower limbs, excessive adipose tissue on the abdomen, on thighs especially, flat umbilical scar, short hands and legs, hyperphagy, dental cavities/ dystrophies, difficult speaking.

Anthropometrical measurements: Weight= 34 Kg (over percentile 95 for age and sex), Waist=105 cm, IMC= 30.9 (over percentile 95 for age and sex, obesity type III), arm circumference= 32 cm, PC= 50 cm, PT= 82 cm, PA= 89 cm.



The clinical aspect of the child leads to PWS (figure 1).

To define the child's state and to evaluate the associated anomalies, there were performed paraclinical investigations and laboratory examinations. Biological tests: GOT=112 U/I, GPT=159 U/I, cortisolemia=540.8 nmol/l, the other laboratory tests being within normal limits. IDR 2 UPPD 0 mm at 72 hours. F.O. normal aspect.

Cardio-pulmonary X-ray: no modifications of pulmonary transparence. Bilateral ascending hemidiaphragm. Enlarged heart in transversal diameter.

Bilateral radiocarpal joint X-ray: deficient bone development – approximately corresponding to the age of 1.6 - 2 years at the level of carpal bones and corresponding to the chronologic age (3 years) at the level of metacarpal bones.

Sella turcica X-ray: no visible bone modifications.

Normal echographic aspect of the abdominal organs. Endocrinologic examination: Obesity level III. Pilary virilism. Neurological examination: psycho-motor retard.

The evolution was favorable under treatment – she was discharged home cardio-respiratory balanced, with recommendations regarding: monitoring the weight, imposing a diet and a physical exercise program (which are absolutely necessary in the context of hyperphagy); neuro-motor recovery, the speaking deficiencies must be evaluated and an educational program must be imposed in order to improve the behavioral status.

The diagnosis clinical suspicion must be followed by the cytogenetic study for the identification of a deletion or of some chromosomal re-arrangements which involve the region 15q11.2-q13, confirmed by the DNA study–molecular analysis (Institute of Clinical Genetics within the Olga Hospital in Stuttgart). The child presents a deletion of the paternal band at the methylation test, thus confirming the clinical diagnosis of PWS.

DISCUSSIONS

The syndrome was described by Prader and collab. in 1956[2,3] 70% of the patients present a deletion of the paternal chromosome which involves the critical region (Myers, Carrel, Whitman, & Allen, 2000). 1% presents a chromosomal re-arrangement with a breaking point in the interval 15q11.2-q13 (Figure 2); 25% of the patients have a monoparental disomy 15 maternal. The human genome is characterized by the existence of some imprinting areas, the expression of the genes in these regions depending on their parental origin. Several genes are known in the imprinting region 15q11.2-q13, but the precise cause of PWS has not been clarified yet.[6]

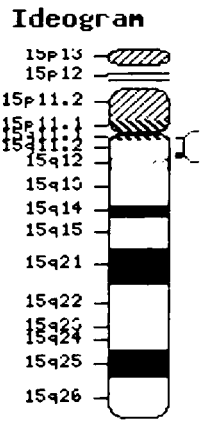


Figure 2. NCBI The OMIM Gene Map

Suggestive symptoms of PWS: severe hypotony during infancy, feeding difficulties in the first months of life, excessive feeding in childhood, gradually developed obesity, cognitive deficiencies, particular behavioral phenotype, hypogonadism and low stature. The clinical diagnosis is strengthened by the particular phenotype of PWS.[4,6]

The diagnosis clinical suspicion must be followed by cytogenetic studies. In their absence, on the basis of clinical criteria, we suggest molecular tests: FISH test, quantitative PCR, maternal UPD, imprinting defects (in patients where there was no identification of a deletion). 95% of the defects of the imprinting centre are micro-deletions of the SNRPN gene, very few patients being identified as presenting other defects. First of all, we recommend the methylation test, followed by the FISH evaluation with a DNA probe which should include the SNRPN gene. A negative FISH test does not exclude the diagnosis and the case also requires the UPD evaluation by means of a micro-satellite analysis.[2,6]

The presented case fulfills the conditions of PWS: during infancy – difficult feeding, muscular hypotony; late physical and psychomotor development on age periods; suggestive clinical examination (hyperphagy, obesity level III, neuropsychic retard, hypogenitalism), confirmed by the DNA methylation analysis–deletion of the paternal band.

The prenatal diagnosis is indicated for the couple with a child affected by imprinting defects duet o the high recurrence risk of 25%.[4,6] The parents of the child with PWS are healthy. The recurrence risk depends on the causal mechanism of the syndrome. Most affected individuals do not have children, but the risk for an affected child depends on the cause of parental affectation and on the sex of the affected parent. In the case of deletion, the risk of recurrence is of 50% for PWS if the father is affected and the risk for Angelman syndrome if the mother is affected.[6]

The differential diagnosis is made with the congenital hypotonies of other cause, with obesity states: Frohlich adiposogenital dystrophy, Albright hereditary osteodystrophy, Laurence-Moon-Biedl syndrome, craniopharingioma, Cohen syndrome.[3]

The treatment of the children with PWS must be adapted to their age. During infancy – special techniques of breast-feeding, gavage feeding (necessary for ensuring an adequate nutrition). Physical therapy can also improve the muscular status. Monitoring weight, imposing a diet and of a physical exercise program; the speaking deficiencies must be evaluated. During childhood, the growth hormone therapy can bring weight to normal, but it requires supervision and monitoring the possible respiratory complications.[1] Calcium therapy is efficient in order to prevent osteoporosis. Children with PWS have a higher rate of mortality because of obesity and its complications. Avoiding obesity by means of early imposed diet and physical exercise program, ever since first childhood, can lead a normal longevity.[6]

CONCLUSIONS

1. The presented case was diagnosed with PWS on the basis the clinical aspect (obesity, obvious muscular hypotony since infancy, neuropsychic retard, hypogenitalism) and was confirmed by a DNA analysis – deletion of the paternal band at the methylation test.

2. In order to clarify the exact cause (paternal deletion, maternal UPD, imprinting defects) and to define the recurrence risk in additional children, we suggest a micro-satellite analysis which should include DNA samples from both parents.

3. The identification of a translation of the imprinting centre or of a translocation in a patient and one of the parents must be followed by offering this option for testing/ genetic advice to the brothers of the bearing parent, too.

4. The treatment aims at limiting polyphagy, monitoring weight, imposing a diet, some physical exercise program, and an educational program which should improve the behavioral status.

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Nitrite determination - degradation product of nitric oxide in decomposition media of nitric oxide sources

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New, previously unknown pharmacological effects of nitric oxide are often published in scientific journals. Thus, new molecules able to generate nitric oxide in vivo are searched. Detection of nitric oxide is very difficult due to its very short half-life. Consequently measurement of nitrite ion, as decomposition product of nitric oxide, is generally accepted. So far there are no simple, fast, inexpensive and highly specific methods for detection of nitric oxide in complex matrices. In our paper we describe a new HPLC method with detection in the visible spectrum based on the well known reaction between nitrite, sulfanilic acid and α-naphtylamine with formation of a red azo dye. This compound can be detected at 520 nm. This method is very simple, fast (an analysis takes only 6 minutes), highly specific and with a very good limit of quantification: 5 ng/ml. We successfully used the method for measuring nitrite amounts generated by nitrones kept under UV light.

***Key words**: nitrite, HPLC, Griess reaction*

More and more biological activities of nitric oxide are discovered since the 1980s when some scientists spotted its importance in mammalian bodies. The best studied effect is the vasodilator one, but other effects are known too: neurotransmitter, role in immunity, inflammation, skin and muscle function, etc. Therefore, substances able to generate nitric oxide will find a place in the modern therapy for other properties too. Because nitric oxide is a very unstable substance (half life varying from seconds to minutes, depending on the matrix) its amounts are very difficult to measure.

One of the best methods for measuring free radicals (nitric oxide being a very active free radical) is the electron spin resonance spectroscopy (ESR), but its low ability to detect nitric oxide (usual complexes obtained with nitric oxide have very large absorption band) makes it difficult to detect biological active concentrations of picomolar levels.[6] Another option would be using chemiluminescence methods that measure nitric oxide as a gas resulted from a solution. Because of its small and instable mass, spectrometry is not a good option. Thus, nitric oxide release from different substances is evaluated by measuring the nitrite levels in the media.

There are several methods available for this purpose: spectrophotometric methods using Griess reagent[3] (high limit of detection: above 100 ng/ml), fluorimetric method that uses the reaction between nitrite and 2,3-diaminonaphtalen (low limit of detection and low specificity in complex matrices)[4], capillary electrophoresis (detection limit is 25 ng/ml)[5], high performance liquid chromatography (HPLC) using the ion-pair technique levels of 10-50 ng/ml nitrite (less specific in complex matrices)[7], gas chromatography coupled with nitrogen phosphorus detector (poor limit of detection, i.e. 100 ng/ml, needs derivatization)[1], gas chromatography coupled with mass spectrometry that needs a derivatisation and expensive equipment (good detection limit, i.e. 4 ng/ml; best specificity).[2]

Our aim in this study was to develop a low cost, high-specificity and high-sensitivity method for measuring nitrite release from donors in complex matrices. Such a method was missing so far.

MATERIAL AND METHODS

- Merck HPLC system: quaternary pump L-7100, auto sampler L-7200, column thermostat L-7360, DAD detector L-7455, interface L-7000, solvent degasser L-7612, and HMS manager software, LiChroCART 125-4 LiChrospher 100 RP-18 (5 μm) Merck column.

- Reagents: sodium nitrite pa, TBA 20% aqueous solution Merck, gradient grade acetonitrile and methanol Merck, phosphoric acid pa Merck, Griess A reagent (50 mg suphanylic acid, 1.5 ml acetic acid, and 3 ml water), Griess B reagent (4 mg α-naphtylamine and 4 ml acetic acid).

Method: - mobile phase: TBA 15 mM in purified water was adjusted to pH 2.5 with phosphoric acid: methanol: acetonitril (54:25:21).

-debit: 1.0 ml/min

-monitored wavelengths: 200-600 nm with the extraction of best chromatogram at 520 nm

-injected volume: 100 μl with loop method

-50 μl of Griess A and 50 μl of Griess B reagents were added to 500 μl of nitrite-containing sample, the injection was made after at least 30 minutes.

-Brukner Daltonics MALDI MS mass spectrometer, α-cyano 4-hydroxy cynamic acid was used as matrix.

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RESULTS AND DISCUSSION

The following parameters of the new method were determined:

- specificity: injection of water, Griess A reagent, Griess B reagent, mixture of the two reagents, nitrite, or any of the decomposed nitrones (nitric oxide source were kept several times under a powerful UV lamp) did not lead to any interference with the studied azo dye. Even when decomposition products were colored no interference was observed (Figure 1). Peak purity was determined for the visible domain (threshold was set to 95%).

In about 10% of the randomly assigned samples the first and second derivatives of peaks were obtained and compared with that of the etalon. In all cases two maxima and one valley were obtained suggesting that no coeluting peak was present (Figure 2).

Using MALDI MS mass spectrometer we proved the formation of the proposed derivative (azo dye). It can be seen that as expected, the 327 M/Z appears in the mass spectra of a mixture of nitrite, Griess A and Griess B reagents. In figure 3 mass spectra of the nitrite-reagents mixture and MS-MS spectra of the 327 M/Z ion are presented.

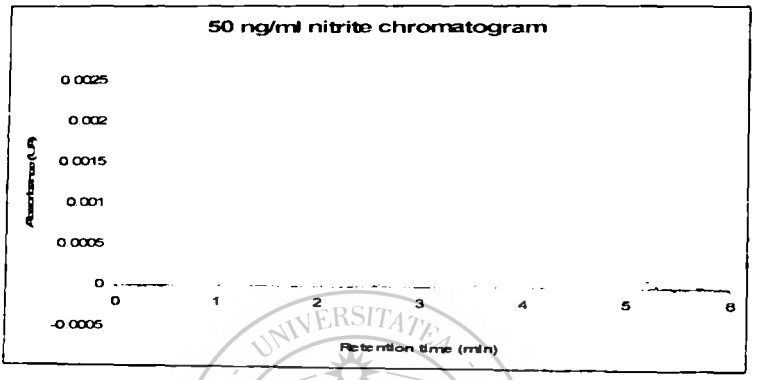

Figure 1. Chromatogram of a 50 mg/ml nitrite solution

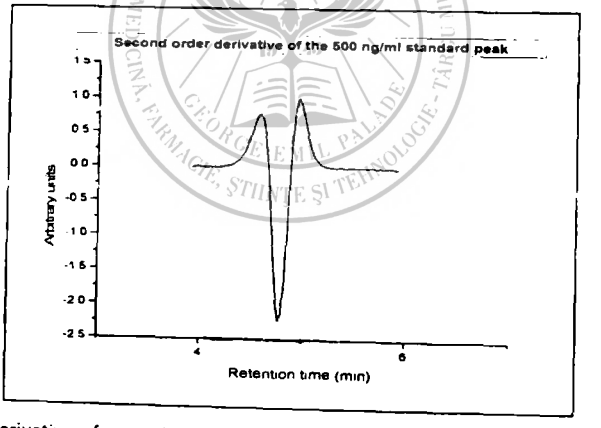

Figure 2. Second order derivative of an etalon solution and a solution of a nitrone kept under UV light for 2 hours

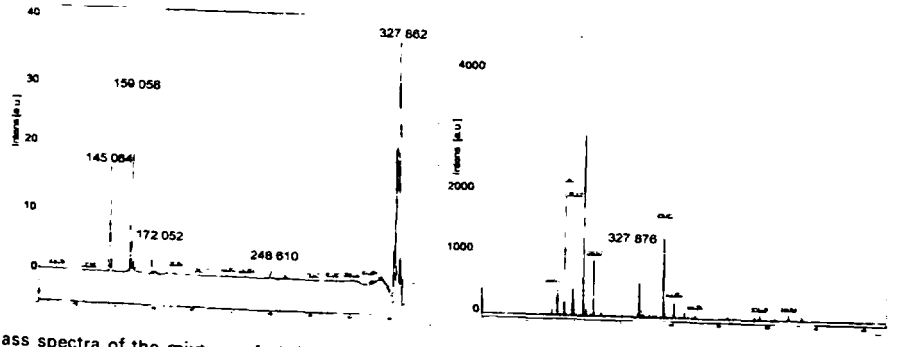

Figure 3. Mass spectra of the mixture of nitrite solution and Griess reagents (right) and MS-MS spectra of the 327 M/Z (left) ion corresponding to the expected azo dye

- linearity: a good linearity was obtained for nitrite in the 0 – 2000 ng/ml range. Most of the residuals (difference between the calculated concentration - from the calibration curve - and the theoretic concentration expressed as a percent of the theoretic concentration) were under 7%, and no consistent increasing or decreasing tendency of the residuals with the concentration was present. Square of the coefficient of correlation was 0.996 (Figure 4).

- intraday and interday coefficient of variation were less than 5%

- accuracy was 97.2-103.7% in aqueous solution

- stability: the formed derivative was stable for at least 24 hours without significant decrease of concentration. The maximum peak intensity was attained after 30 minutes.

- limit of quantification is 5 ng/ml

- limit of detection: not determined

The method was successfully used for measuring the nitrite amounts resulted from the decomposition of nitrones (induced by UV light) in aqueous matrix (Figure 5).

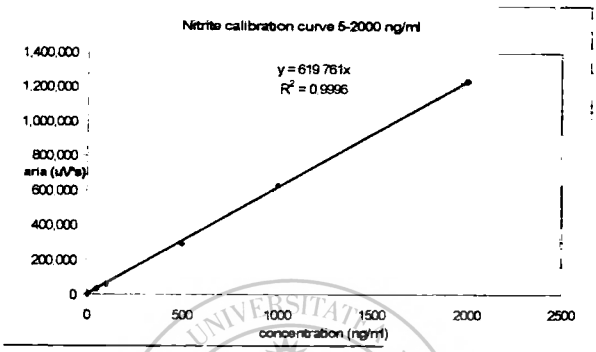

Figure 4. Nitrite calibration curve

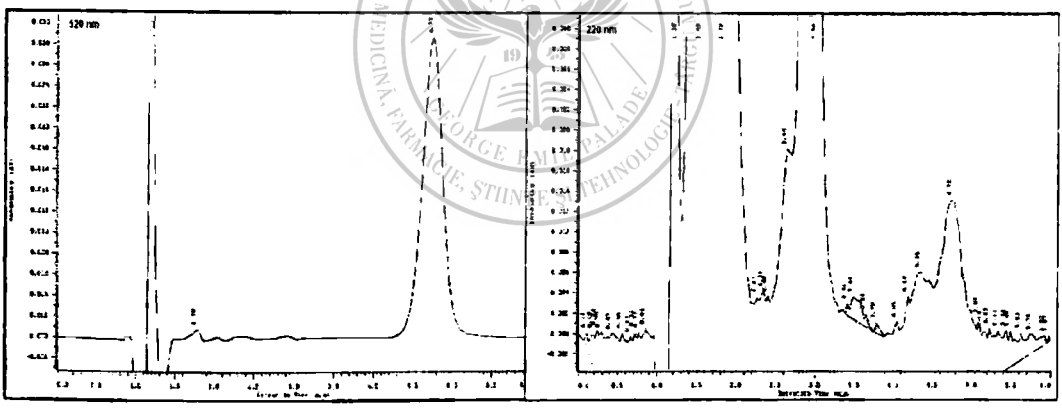

Figure 5. Two chromatograms at different wavelengths of the same decomposed nitrone sample: 520 nm (left) and 220 nm (right)

Compared to other methods, with similar or higher detection limit[3,4,5,7], our method is able to detect nitrite in complex matrices with lower probability of interference. For example the HPLC ion-pair technique reaches detection limits of µg/ml when used in complex matrices.

This widely employed HPLC-UV method based on ion pair formation[7] was impossible to be used for our complex matrices because of the high UV absorption abilities of the nitrones and decomposition products. These products elute in the same region where nitrite elutes making impossible to read low nitrite concentrations. Furthermore, we were not sure of the identity and purity of peaks since nitrones and their decomposition products have similar absorption spectra to nitrite. For comparison we presented an UV chromatogram together with the visible one (Figure 5).

The derivatization process we used is very simple and needs no further extraction or sample treatment. The analysis time is also very advantageous: it takes only 6 minutes. The formation of the proposed derivative was confirmed with MALDI mass spectrometry.

CONCLUSIONS

We report a new method for determination of nitrite in various matrices. Instead of the colorimetric method (known since 1858 and still used), the HPLC-

UV-VIS one brought extra specificity and also lowered the detection limit at least 30 times.

Good results were obtained in determining nitrite form the decomposition solutions of some nitrones with various structures.

The only disadvantage of our method at present is the impossibility of determining nitrate which is a decomposition product of nitrite. However, there are many methods to reduce nitrate to nitrite, which moderates this handicap. Determining nitrate too would not be a time issue since the required two analyses take maximum 12 minutes by the HPLC method.

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Simultaneous quantitative determination of amiodarone and its active metabolite: desethylamiodarone in human plasma by LC-MS method

Réka Borka-Balás[1], Szende Vancea[2], Mária Nyulas[1]

Amiodarone is a class III antiarrhytmic drug. In therapy its potent effectiveness can lead to strong negative side effects, so regular monitoring of amiodarone and its active metabolite: desethylamiodarone serum levels is essential. The aim of this study was to develop a rapid and sensitive LC-MS method for the routine analysis of amiodarone and desethylamiodarone in plasma samples. Amiodarone (AMI) and desethylamiodarone (DEA) human plasma samples were precipitated with methanol. Before centrifuging at 14000 rpm for 10 min the mixture was vortexed for 30 seconds. 450 µl of supernatant was transferred in chromatographic vials and 10 ml was injected into LC-MS. Column: Zorbax SB-C18 (Agilent Technologies). Eluent: Formic acid 0.1%: Acetonitrile 45:55. Analysis time: 11.5 min. Minimum quantification limit (LLOQ) was 100 ng/ml for amiodarone and for desethylamiodarone too. Quantitation was performed by peak area method.

***Key words:** amiodarone, desethylamiodarone, analythical method, mass spectrometry*

Amiodarone has a well established role in the treatment of ventricular tachyarrhythmias. Although considered to be a class III anti-arrhythmic, amiodarone also has class I/b, II and IV actions, which gives it a unique pharmacological and anti-arrhythmic profile.[1] Amiodarone is a structural analogue of thyroid hormone and some of its anti-arrhythmic properties and toxicity may be attributable to interactions with nuclear thyroid hormone receptors. The lipid solubility of amiodarone gives it an exceptionally long half-life.[5] Oral amiodarone takes days to work in ventricular tachyarrhythmias, but iv. amiodarone has immediate effect and can be used in life threatening ventricular arrhythmias.

Amiodarone is effective in chronic and recent onset atrial fibrillation and orally or iv. for atrial fibrillation after heart surgery, in controlling refractory tachyarrhythmias in infants and is safe after cardiac surgery.[16,12] In therapy its potent effectiveness can lead to strong negative side effects, so regular monitoring of amiodarone and its active metabolite: desethylamiodarone plasma levels is essential.[14,15,8]

Several analytical methods for determination of amiodarone and desethylamiodarone concentration in human plasma have been reported, but usually liquid-liquid or solid-phase extraction is necessary in order to concentrate or sample clean-up, these sample treatments supposing a time consuming work.

LC-MS has been widely accepted as the main tool in the identification, structure characterization and quantitative analysis of drugs and its metabolites owing to its superior sensitivity, specificity and efficiency. The analysis must be carried out in complex biological matrices and therefore efficient sample preparation and liquid chromatography are often required to achieve good specificity of the analysis. LC-MS grows in popularity, despite the equipment costs, and in the case of simultaneous amiodarone and desethylamiodarone determination in plasma there are only a few published articles[18,19,10,11] and in all cases the sample clean-up and concentration by liquid-liquid or solid phase extraction were applied.

The aim of this study was to develop a rapid and sensitive LC-MS method for the routine analysis of amiodarone and desethylamiodarone in plasma samples applying a simple protein precipitation.

MATERIALS AND METHOD

Standards: Amiodarone: Molecular formula: $C_{25}H_{29}I_2O_3$, Molecular weight: 681.78

Desethylamiodarone: Molecular formula: $C_{23}H_{25}I_2NO_3$ Molecular weight: 617.26

Blank plasma has been obtained from healthy volunteers and used for the preparation of calibration,

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quality control (QC) and spiked samples. Individual blanks from eight volunteers have been obtained for the investigation of specificity. EDTA has been used for anticoagulation. Reagents: Methanol, Acetonitrile, Purified water. Instruments: Agilent 1200 Series: HPLC , LC/MSD Trap SL: Agilent 1100 Series.

Amiodarone calibration stock solutions:

-10.6 mg amiodarone hydrochloride was dissolved in 10 ml methanol, Code: ST-AMI-1000, Concentration: 1000 µg/ml amiodarone

-1 ml ST-AMI -1000 will be dissolved in 10 ml methanol, Code: ST-AMI –100, Concentration: 100 µg/ml amiodarone

-1 ml ST-AMI -100 will be dissolved in 10 ml purified water, Code: ST-AMI -10, Concentration: 10 µg/ml amiodarone

Desethylamiodarone calibration stock solutions:

-10 mg desethylamiodarone hydrochloride was dissolved in 10 ml methanol, Code: ST-DEA-1000, Concentration: 1000 µg/ml desethylamiodarone

-1 ml ST-DEA -1000 will be dissolved in 10 ml methanol, Code: ST-DEA –100Concentration: 100 µg/ml desethylamiodarone

-1 ml ST-DEA-100 will be dissolved in 10 ml purified water, Code: ST-DEA -10, Concentration: 10 µg/ml desethylamiodarone

Ultrapure water for LC was used as solvent for the dilutions (Table I).

Calibration standards have been freshly prepared on the day of analysis. 40 µl of the appropriate working solution were added to160 µl of blank plasma (Table II).

QC samples: 40 µl of the appropriate working solutions were added to 160 µl blank plasma (Table III).

QC samples were stored frozen below –20 °C or

Table I. Calibration working solutions

Calibration working solutions				
Added solution		Diluted working solution		
Code	Volume (ml)	Volum (ml)	Concentration(ng/ml)	Code
ST-AMI-10 ST	0.5	10	500	W--AMI+DEA-500
ST-DEA-10	0,5		500	
ST-AMI-10	1,0	10	1000	W_2-AMI+DEA-1000
ST-DEA-10	1,0		1000	
ST-AMI-100	0,2	10	2000	W_3-AMI+DEA-2000
ST-DEA-100	0,2		2000	
ST-AMI-100	0,3	10	3000	W_4-AMI+DEA-3000
ST-DEA-100	0,3		3000	
ST-AMI-100	0,4	10	4000	W_5-AMI+DEA-4000
ST-DEA-100	0,4		4000	
ST-AMI-100	0,5	10	5000	W_6-AMI+DEA-5000
ST-DEA-100	0,5		5000	
ST-AMI-100	0,7	10	7000	W_7-AMI+DEA-7000
ST-DEA-100	0,7		7000	
ST-AMI-100	1,0	10	10000	W_8-AMI+DEA-10000

Table II. Calibration standards

Code of added working solution	Calibration standard		
	Code	Concentration (ng/ml)	
		AMI	DEA
W_1	STD1	100	100
W_2	STD2	200	200
W_3	STD3	400	400
W_4	STD4	600	600
W_5	STD5	800	800
W_6	STD6	1000	1000
W_7	STD7	1400	1400
W_8	STD8	2000	2000

Table III. QC samples

Code of added solution	QC sample		
	Code	Concentration (ng/ml)	
		AMI	DEA
W_A	QCA	300	300
W_B	QCB	1200	1200
W_C	QCC	1600	1600

less until use. Sample preparation: 200 µl plasma sample was transferred into polypropylene tubes. 0.3 ml methanol was added for protein precipitation. Samples were shaken 30 seconds using vortex mixer then centrifuged for 10 minutes at 14000 RPM. 450 µl of supernatant was transferred into chromatographic vial.The injection volume was 10 µl. HPLC system: Agilent 1200 Series HPLC, Column: Zorbax SB-C18, Eluent: Formic acid 0.1%: Acetonitrile 45:55, Stop time: 10.0 min, Analysis time: 11.5 min, Injection volume: 10 µl. MS detection: Agilent 1100 Series LC/MSD Trap SL, Ionization: ESI, Positive. Determination of concentrations: Chromatograms were processed using QuantAnalysis 5.2 for LC MSD Agilent Technologies software. Concentrations were determined automatically by the instruments data system. Calibrations were performed using singlicate calibration standards.The calibration curve was determined with a weighted least squares analysis.

RESULTS

Validation of the extraction procedure: five QCA, QCB and QCC samples were prepared using the above mentioned extraction method and measured in a single run, against calibration curve. Mean concentrations, deviation of the mean from the nominal value (bias%) and relative standard deviation (percentage coefficient of variation, CV%) of the measured concentrations were calculated for all concentration levels. Accuracy and precision for the extraction procedure lies within the +/-15% relative to nominal concentration and 15% RSD. Selectivity: has been investigated by analyzing eight different blank plasma samples for interference of endogenous compounds with the analytes Investigation of selectivity was carried out together with the calibration curve assay. Mean of the peak areas measured for 100 ng/ml amiodarone and 100 ng/ml desethylamiodarone calibration standard (lower limit of quantification, LLOQ) samples were calculated and used for comparison with possible endogenous peaks in blank samples.[2,3,8,17] Chromatograms of the blank plasma extracts did not exhibit significant peak interferences at the retention times of the examined peaks (Figure 1).

Calibration curves (linearity): eight calibration standards at each concentration level were prepared and measured in a single run. The calibration curves parameters for AMI and DEA in each of the 5 analytical runs are presented in tables IV and V.

Percentage residuals (% difference of the back calculated concentration from the nominal concentration) were calculated for each calibration standards. The residuals were within +/- 20% at the LLOQ and within +/- 15% at all other calibration levels.

Accuracy and precision: In a single validation batch 5 replicate QC samples were analyzed at each of the concentration levels of 300 ng/ml, 1200 ng/ml, 1600 ng/ml amiodarone and desethylamiodarone, against calibration curve (within-run accuracy and precision).

On each of six different days a single QC sample from each of the concentration levels of 300ng/ml, 1200 ng/ml, 1600 ng/ml amiodarone and desethylamiodarone was analyzed, against daily calibration (between-run accuracy and precision).

Mean concentrations, deviation of the mean from the nominal value (bias%) and relative standard deviation (percentage coefficient of variation, CV%) of the measured concentrations were calculated for all concentration levels. At each concentration level (LLOQ, QCA, QCB and QCC) the mean lies within the accepted range of ±15% of the nominal concentration (accuracy) and ±15% of the coefficient of variation.

Characteristics of the method are presented in table VI.

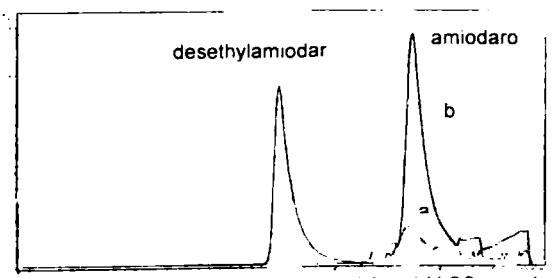


Figure 1. Chromatogram of a blank plasma (a) and LLOQ sample (b)

Table IV. Summary of calibration curves parameters for Amiodarone

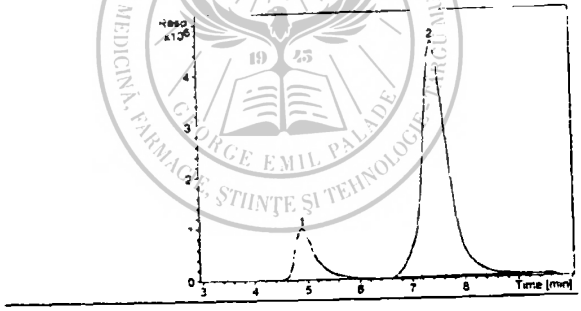
Run	N	A	B	C	Coefficient of determination (R^2)
1	8	-0.415208	143695.378024	-13906788.77187	0.997949
2	8	12.448066	130052.666621	2961394.885899	0.997851
3	8	10.730906	101848.711782	-698686.964657	0.999234
4	8	-3.201802	168412.439457	-7122702.183904	0.991785
5	8	10.987546	100023.363847	-1633441.718463	0.995681
6	8	23.320911	110838.751807	-1808181.910231	0.996529

Table V Summary of calibration curves parameters for Desethylamiodarone

Run	N	A	B	C	Coefficient of determination (R^2)
1	8	20.801339	119008.044039	-13750683.09367	0.997029
2	8	-2.373646	124761.739787	-821856.183367	0.999523
3	8	5.450576	94744.886080	-892874.584463	0.999433
4	8	-1.656332	94165.444389	-3303955.620587	0.998462
5	8	-4.728010	93754.831834	-2800511.187210	0.995281
6	8	2.364658	68398.010809	-567495.073443	0.999666

Table VI. Method validation characteristics

	AMIODARONE	DESETHYLAMIODARONE
Linearity	0.991785-0.999234	0.995281-0.999666
Calibration range	100-2000ng/ml	100-2000ng/ml
Lower limit of quantitation	100ng/ml	100ng/ml
Within-run accuracy	105.48-108.86%	90.93-108.09%
Within-run precision (%rsd)	6.06-8.74%	2.56-3.56%
Between-run accuracy	91.06-101.88%	91.99-100.25%
Between-run precision (%rsd)	0.64-2.06%	3.05-3.37%
Recovery of analyte	47.35-62.81%	53.29-82.72%
Stability in matrix after 3 freeze-thaw cycles	109.5-118.2%	99.57-135.41%
Stability in matrix at room temperature after 6 h	71.13-115.48%	82.07-103.64%
Stability following sample processing after 24 hours	83.1-90.2%	69.67-115.33%



Compound List:

#	Rel.Time	Name	Area	Calc. Conc.	Accuracy
1	4.9	Desethylam-odarone	28247097	415.3 ng/ml	
2	7.4	Amiodarone	15295620	1128.4 ng/ml	

Figure 2. A typical plasma sample chromatogram

Lower limit of quantification: In a single validation batch 8 replicate LLOQ samples were analyzed at the concentration level of 100 ng/ml of amiodarone and 100 ng/ml desethylamiodarone, against calibration curve. On each of six different analytical runs a single LLOQ sample of 100 ng/ml of amiodarone and 100 ng/ml desethylamiodarone was analyzed against daily calibration curve. Mean concentrations, percentage of the mean from the nominal value (accuracy) and relative standard deviation (CV%) of the measured concentrations were calculated. Accuracy and precision for amiodarone and desethylamiodarone lie within the +/-20% relative to nominal concentration and 20% RSD (relative standard deviation) (Figure 2).

Short-term room temperature stability: Five aliquots of QCA, QCB and QCC (BTSL, BTSM and BTSH samples) were allowed to thaw and were stored at room temperature (about 24°C) for 3 hours, than extracted and analyzed together with freshly thawed QC samples of the same concentration and number. Mean concentrations were calculated for the stored and the reference samples and compared. The mean concentration obtained for the stored and the reference samples doesn't differ more than 15% in case of 6 h stored samples, therefore the analytes can be considered as stable in plasma matrix at room temperature for 6 hours. The samples were processed during this 6 hours. Post-preparative stability: Five QCA, QCB and QCC aliquots (OLSL, OLSM and OLSH samples) were subjected to sample preparation. The extracted samples

were left in autosampler at 15°C for 24 hours, and then analyzed together with freshly prepared QC samples of the same concentration. The results indicates that the extracted samples are stable at 15°C in autosampler for at least 24 hours. Recovery of AMI and DEA was evaluated by comparing peak area of extracted samples of low (QCA), medium (QCB) and high (QCC) quality control samples to peak area of appropriately diluted standard solutions at each concentration level. Recovery values are between 47.3 – 62.8 for amiodarone and 53-82.7% for desethylamiodarone.

DISCUSSIONS

The extraction procedure of AMI and DEA from plasma matrix involves a protein precipitation with methanol. The calibration range was chosen from literature data and lies between 100 – 2000 ng/ml. Calibration curves are found accurate and precise over the calibration range and the coefficients of correlation (R^2) are greater than 0.99. Within-run and between-run accuracy and precision results are all within the accepted range (+/-20 at LLOQ and ±15% at QC levels). The recovery of analytes from plasma samples is consistent and constant over the concentration range. The analytes are stable in plasma matrix at room temperature 6 hours and at 15°C following sample processing for at least 24 hours. Both analytes prove stable after 3 freeze-thaw cycles. The results of the validation study indicate that the analytical method for determination of amiodarone and desethylamiodarone in plasma samples is suitable for the bioanalytical-clinical study, for monitorization of therapeutical plasma concentration of amiodarone and desethylamiodarone. In comparison with previously published LC-MS methods [10,11,18,19], the concentration range of the proposed method is higher than in these methods, but it is well enough for determination of therapeutical plasma concentrations.

CONCLUSIONS

The main advantage is the sample preparation by protein precipitation and besides its simplicity, that sample treatment allows obtaining a good recovery of the analyte. The simple sample preparation by protein precipitation, while using less organic solvent with small amounts of sample plasma volume; the relatively short run time and the selected signals for monitoring allowed a specific and efficient analysis of plasma samples, making the method more productive and thus more cost effective.

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Diagnostic possibilities for occlusal caries in permanent teeth

A. Sitaru, A. Monea, Monica Pop

The reduction in caries prevalence has not occurred at the same rate for all dental surfaces, the occlusal sites are still the most likely to develop caries. In order to detect these lesions in early stages, the methods of diagnosis are still being evaluated.
The purpose of this study was to compare laser fluorescence (LF) system DIAGNOdent, with a visual inspection system (VI) for in vitro detection of occlusal caries.
Material and methods We used 27 extracted premolars and molars with macroscopically intact occlusal surfaces, which we examined at an interval of 10 days by a single person using LF and VI methods. The correct diagnosis was made by histological examination.
Conclusions The visual inspection system is more reliable than the laser fluorescence device. DIAGNOdent system should be considered only as an adjuvant for occlusal caries diagnosis.
***Key words**: laser fluorescence, caries*

Despite the fact that the prevalence of dental caries has declined considerably, it is still a problem of great importance. The reduction in caries prevalence has not occurred at the same rate for all dental surfaces and the occlusal surfaces are still the most likely sites for the development of lesions.

Several methods of dental caries diagnosis have been used for more than half a century. Although there are known drawbacks, visual inspection (VI) alone has been claimed to be the best diagnostic method in populations with low caries prevalence, but it is unable to correctly detect caries lesions because of its low sensitivity.[7] On the other hand, the use of a sharp probe along with the visual method does not appear to improve the diagnostic accuracy.[12] It may contaminate other sound sites, damage the fissure[2] as well as facilitate the lesion's progression.

In the search for more accurate diagnostic approaches, investigators have used alternative non-invasive and instrument-based techniques for detecting and quantifying demineralization lesions.[6,9]

A new device is the KaVo DIAGNOdent® (KaVo, Germany), which generates a laser light that is absorbed by both inorganic and organic tooth substances and by metabolites from oral bacteria.[6] In the presence of caries, light with a higher wavelength is re-emitted, known as laser fluorescence effect (LF), and the changes are registered in a digital number scale.

The aim of this study was to validate histologically the use of DIAGNOdent for the detection and quantification of caries on occlusal surfaces; to compare the use of this device with VI.

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MATERIALS AND METHODS

A total of 27 premolars and molars presenting macroscopically intact occlusal surfaces were selected and cleaned to remove any debris. Inclusion criteria for teeth in this study were the apparent absence of occlusal restorations and fissure sealants, absence of hypoplastic pits and frank occlusal cavitation. The teeth were stored in a physiological saline solution before the beginning of the study.

The occlusal surfaces were cleaned and examined by a single person (A.S.), who was trained to use both systems. The scoring for visual inspection is presented in Table I.

After removing each tooth one-by-one from the saline solution, the sites were examined under a standard dental operating light at an eye-tooth distance of 20 cm. If no visible signs were seen on the wet occlusal surface, the examiners were allowed to dry the teeth with compressed air.

The measurements with the LF device were made after calibration of the device with the ceramic standard. The assessment of the teeth with the LF fiber-tip was performed according to the distributor's instructions. The laser tip (A tip) was positioned on a sound enamel region to provide a baseline measurement. After that, the laser tip was positioned on the target site and rotated around its long axis; the highest value was then recorded. To verify intra-examiner reproducibility the examiner re-performed all examinations after a period of 7 to 10 days.

Table I. Criteria used for visual and histological examination (Ekstrand et al. 1997)

Score	Visual	Histological
0	No or slight change in enamel after prolonged air-drying	No enamel demineralization
1	Opacity or discoloration hardly visible on the wet enamel, but distinctly visible after air-drying	Enamel demineralization limited to the outer 50% of the enamel layer
2	Opacity or discoloration in enamel distinctly visible without air-drying	Demineralization involving between 50% of the enamel and 1/3 of dentine
3	Localized enamel breakdown in opaque or discolored enamel and/or grayish discoloration from the underlying dentine	Demineralization involving the middle 1/3 of dentine
4	Cavitation in opaque or discolored enamel exposing dentine	Demineralization involving the inner 1/3 of dentine

In order to consider a lesion as "carious", for visual inspection system, the threshold was established between 1 and 2 (Table I), and for LF device it was set between D1 and D2 (Table II).

Table II. Ranked scale used in DIAGNOdent examination

Method	Histological exmination	VI	LF
Carious lesion %	30,76	25	40,38
Error range	-	-18,75%	+31,25%

Histological examination was done by the same person, after all teeth were sectioned in a buccal to lingual direction and then examined with a surgical microscope. Any alteration of enamel or dentin was recorded according to the criteria of the visual system.

RESULTS

Histological examination showed that 21 sites were classified as score 0; 15 as score 1; 8 as score 2; 5 as score 3 and 2 as score 4 (Figure 1). Hence, 16 out of 5 sites were classified as "carious", which represents 30,76% of the samples.

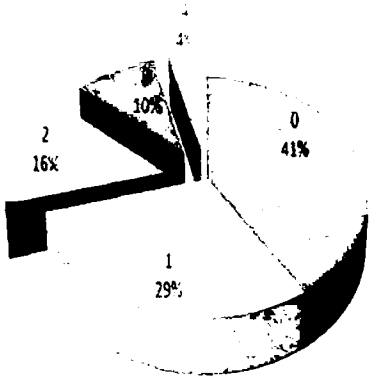

Figure 1. Histological examination scores

Visual inspection examination showed the following results: 27 zones as score 0, 13 as score 1, 7 as score 2, 4 as score 3 and 1 as score 4. From the total of 52 zones, 13 were classified as caries, which represented 25% of the samples. (Figure 2)

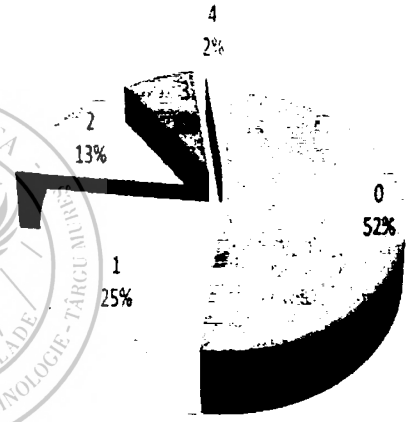

Figure 2. Visual inspection scores

DIAGNOdent measurements gave the following results: 31 as D1, 12 as D2 and 9 as D3, which means 40,38% of the samples are carious. (Figure 3)

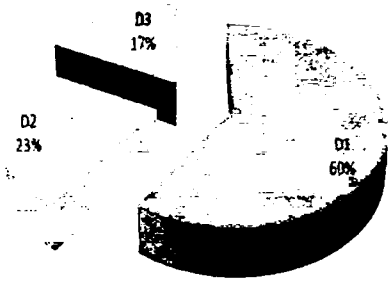

Figure 3. DIAGNOdent readings

The found results of the carious lesions using the two systems of investigation were compared with the histological data in Table III.

Table I. Comparison between VI, LF and histological examination results for caries

Method	Histological exmination	VI	LF
Carious lesion %	30,76	25	40,38
Error range	-	-18,75%	+31,25%

Comparing true and false readings in each of the two systems, we came to the conclusion that VI system shows lower sensitivity, but higher specificity than the LF method.

Figure 4. Sensitivity and specificity for VI and LF systems

DISCUSSION

The VI has been considered an invaluable tool in the last decades. Due to its high specificity, this diagnostic method avoids overtreatment, mainly in low caries prevalence populations. However, only recently, the VI has also demonstrated high sensitivity values[13], which is important to avoid undertreatment. This has been due to the employment of the VI proposed by Ekstrand et al.[3] (1997). These authors demonstrated, in a laboratory setting, a good correlation between occlusal signs and the histological depth of the lesion, which implies that the poor sensitivity values of other workers' VI could be attributed to a failure in selecting appropriate VI criteria.

The LF device can be considered an adjuvant tool for occlusal caries diagnosis. However, this system cannot be used as a substitute for VI, since its sole use could lead to overtreatment. According to the Ekstrand's visual system[3], the presence of discoloration, visible without air-drying, is an indicator of demineralization involving between 50% of the enamel and the outer 1/3 of dentine. Thus, all sites presenting this visual pattern are considered sound by the visual inspection, while for the LF diagnostic method there is a high tendency of being considered caries-affected, which results in a high rate of false-positive answers. Recent evidence has shown that the LF device tends to overscore discolored sites.[4,5,14] (Table III).

Although some researchers have detected a good to excellent performance of the LF device[8,9,10,13], others have not observed this trend. Several factors may account for this difference, such as the recommended cut-offs for interpretation of the LF measurements[5] and the storage medium of the sample before the beginning of the study. If we carefully examine the best cut-off points used for LF readings in clinical studies, one should observe that values higher than 19-20 indicate dentinal involvement and are currently used as a threshold between "sound" and "carious" sites[1,10,15], which is in agreement with the present investigation. Therefore, when LF readings were higher than 20, it was likely that the lesion has extended into dentine. One should bear in mind, however, that the use of this criterion could lead to overtreatment in discolored sites.

CONCLUSION

A meticulous VI enables the dentist to score earliest signs of caries changes or to differentiate between fissures with or without discoloration, staining or opacities. The LF showed a good performance on occlusal caries diagnosis. However, due to its relative high cost and performance inferior to that obtained through the visual criteria, it should be considered only as an alternative method for occlusal caries diagnosis.

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