Poznan University of Medical Sciences
Poland

JMS Journal of Medical Science

previously Nowiny Lekarskie

Founded in 1889

2015
Vol. 84, No. 3

QUARTERLY

Indexed in:
Polish Medical Bibliography, Index Copernicus,
Ministry of Science and Higher Education, Ebsco, Google Scholar

eISSN 2353-9801
ISSN 2353-9798

www.jms.ump.edu.pl

SECRETARIAT ADDRESS
70 Bukowska Street, room 104
60-812 Poznan, Poland
phone/fax: +48 61 854 72 74
email: nowinylekarskie@ump.edu.pl
www.jms.ump.edu.pl

DISTRIBUTION AND SUBSCRIPTIONS
37a Przybyszewskiego Street
60-356 Poznan, Poland
phone/fax: +48 61 854 64 87
email: sprzedazwydawnictw@ump.edu.pl

PUBLISHER
Poznan University of Medical Sciences

eISSN 2353-9801
ISSN 2353-9798

Publishing Manager: Grażyna Dromirecka
Technical Editor: Bartłomiej Wąsiel

WYDAWNICTWO NAUKOWE UNIWERSYTETU MEDYCZNEGO IM. KAROLA MARCINKOWSKIEGO W POZNANIU
60-812 Poznań, ul. Bukowska 70
tel./fax: +48 61 854 71 51

Ark. wyd. 7,8. Ark. druk. 9,0.
Zam. nr 1/16.

The Editorial Board kindly informs that since 2014 *Nowiny Lekarskie* has been renamed to *Journal of Medical Science*.

The renaming was caused by using English as the language of publications and by a wide range of other organisational changes. They were necessary to follow dynamic transformations on the publishing market. The Editors also wanted to improve the factual and publishing standard of the journal. We wish to assure our readers that we will continue the good tradition of *Nowiny Lekarskie*.

You are welcome to publish your basic, medical and pharmaceutical science articles in *Journal of Medical Science*.

Ethical guidelines

The Journal of Medical Science applies the ethical principles and procedures recommended by COPE (Committee on Conduct Ethics), contained in the Code of Conduct and Best Practice Guidelines for Journal Editors, Peer Reviewers and Authors available on the COPE website: https://publicationethics.org/resources/guidelines

CONTENTS

ORIGINAL PAPERS

Adam Dreksler, Marcin Lenkowski, Michał Marcinkowski, Marian Tomasz Nowaczyk, Krzysztof Osmola, Tomasz Pełczyński, Gleb Radzewicz, Samir Rahma

A 10 year data analysis of lymphatic system metastasis and recurrence in individuals affected by malignant tumors within the oral cavity and maxillofacial region 141

Piotr Eder, Liliana Łykowska-Szuber, Iwona Krela-Kaźmierczak, Kamila Stawczyk-Eder, Katarzyna Klimczak, Aleksandra Szymczak, Krzysztof Linke

Safety profile of anti-tumor necrosis factor therapy in inflammatory bowel disease – a single center experience 146

Małgorzata Schlabs, Katarzyna Pawlak-Buś, Piotr Leszczyński

Retrospective analysis of infections prevalence in patients with progressive systemic sclerosis treated with cyclophosphamide 152

Witold Woźniak, Małgorzata Grzymisławska, Joanna Łupicka, Małgorzata Bruska, Adam Piotrowski, Anna Gałązka, Magdalena Rojewska, Jarosław Sobański

Early differentation of lamellar structure of the intervertebral disc in staged human embryos 157

Ewelina Swora-Cwynar, Jacek Karczewski, Anna Musiał, Marian Grzymisławski, Emilia Marcinkowska, Agnieszka Dobrowolska, Dorota Mańkowska-Wierzbicka

Assessment of nutritional status and feeding methods in patients with inflammatory bowel disease 167

Wiktor Suchy, Agnieszka Gaczkowska, Adam Pawelczyk, Piotr Pukacki, Robert Chudzik

Alcohol use among medicine and law students in Poland 183

Radosław Tymiński, Michał Walczewski, Michał Wieczorek

Identifying patients' criteria for assessment of doctors on Polish physician rating websites 189

REVIEW PAPERS

Anna Marcinkowska-Gapińska, Piotr Kowal

Hemorheological studies of chosen clinical cases 197

Jakub Żurawski

Thin basement membrane disease – literature review 201

Instructions for Authors 205

ORIGINAL PAPER

DOI: https://doi.org/10.20883/medical.e10

A 10 year data analysis of lymphatic system metastasis and recurrence in individuals affected by malignant tumors within the oral cavity and maxillofacial region

Adam Dreksler[1], Marcin Lenkowski[1], Michał Marcinkowski[2], Marian Tomasz Nowaczyk[2], Krzysztof Osmola[2], Tomasz Pełczyński[1], Gleb Radzewicz[1], Samir Rahma[1]

[1] Student Scientific Circle in Department of Maxillofacial Surgery, Poznan University of Medical Sciences, Poland
[2] Department of Maxillofacial Surgery, Poznan University of Medical Sciences, Poland

ABSTRACT

Introduction. Tumors are only second to cardiovascular diseases as a cause of death. According to World Health Organization (WHO), these statistics will increase especially within aging and well-developed countries.
Aim. The aim of the research was to analyze the frequency of recurrences and lymph node involvement in individuals affected by malignant tumor of head and neck region. Special attention was paid to tumor location, sex, age of the affected individuals as well as histological appearance of the tumor.
Material and methods. The research team has analyzed medical data record base from 2002–2011 and selected a group of 1697 individuals treated for malignant tumors within the oral cavity and maxillofacial region.
Results. Reoperation was carried out on 192 patients due to recurrences and lymph node involvement; (11.3%) of the study group. Patients were further subdivided into groups according to sex, age, histological appearance and anatomical location of tumor recurrence. Results were compared with the available literature.
Conclusions. Our data confirms the findings of other research groups in terms of accuracy of early diagnostic screening and its effect on final tumor treatment. It is also essential to accentuate the importance of post-treatment monitoring within first five years after initiation of treatment, which allows for an early determination of local recurrences and/or lymph node involvement.

Keywords: tumors of head, tumors of neck, recurrence.

Introduction

Tumors are only second to cardiovascular diseases as a cause of death. According to World Health Organization (WHO), these statistics will increase especially within aging and well-developed countries. In 2010, United States reported 36000 new cases of cancers involving the regions of oral cavity and head and neck. 90% of these carcinomas were diagnosed histologically as squamous cell carcinomas (SCC) [1]. Data acquired in Poland also shows an increasing trend of oral cancer occurrence. In 1963 there were 35328 new cases, in 1990, 83470, whereas in 2003, 121444, which is equivalent to an increase of risk by 3.43 times. In the region of Great Poland (Wielkopolska), situated in the western part of Poland, in 1999 there were 10316 new malignant cases, whereas in 2009, there were 12966 new cases (6554 men, 6412 women) which is equivalent to increase of 25% (2650). In 2009, the number of fatalities reported was 8258. There has also been an increase in the number of tumors in the oral and head and neck region. In 1999, the Great Poland registered 815 new cases involving head and neck area, whereas in 2009, the number of 909 new cases [2]. The most

common head and neck cancer is SCC. Despite the fact that there has been an improvement in SCC treatment, the number of post-treatment complications is still significant. Patients negligence in the early stages of cancer is the main reason for unfavorable prognosis and often leads to a high degree of tumor involvement. Data indicates that there has been a substantial increase in the number of head and neck carcinomas and unfortunately this unfavorable trend is expected to create a major social and medical burden.

Aim

The aim of the research was to analyze the frequency of recurrences and lymph node involvement in individuals affected by malignant tumor of head and neck region. Special attention was paid to tumor location, sex, age of the affected individuals as well as histological appearance of the tumor.

Material and methods

A particular group of individuals has been selected based on medical data record base from 2002–2011 and selected a group of patients operated due to tumors within the oral cavity, skeletal region of the maxilla and mandible as well as skin covering of the head and neck. Further analysis has been performed on a group of individuals on which reoperation was performed due to recurrence within the primary site or due to regional lymph metastasis. Patients were subdivided into groups according to sex, age, histological appearance and anatomical location of tumor recurrence.

Results

According to medical data from ten years period from 2002–2011, 1697 individuals (612 females, 1085 males) were operated due to malignancy. The female/male ratio was (F/M) 1/1.77. The age of patients ranged from 9 to 96 years. The most common location of primary lesion of the tumor has been located in the floor of the mouth or alveolar part of mandible. Detailed location of primary lesions has been shown in **Figure 1**. Histological analysis of oral mucosa showed SCC as the most commonly occurring carcinoma (**Table 1**).

The reason for the reoperation during analyzed period of time was the process of local recurrence and metastasis to the regional lymph nodes in 192 patients (69 females, 123 men), (F/M = 1.78) (**Figures 2, 3**). The age of re-operated patients varied from 21 to 90 years. The most numerous group consisted of patients aged from 51–60 years (**Table 2**).

The greatest number of recurrences was found in patients in which primary lesion was located in maxillary and palatal region (39.1%). The least commonly reoperated individuals presented with a primary lesion within soft tissues (gingival tissue, buccal vestibule and skin from unspecified parts of the face) and lymph nodes of the oral cavity region. 75.5% of recurrences occurred within the first 2 years. The average time of

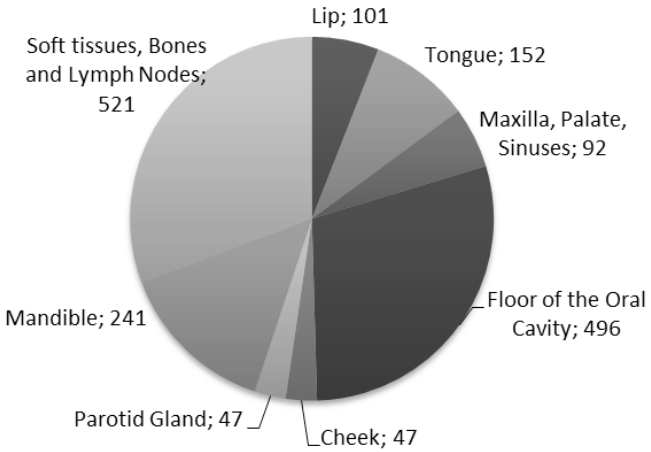

Figure 1. Primary location of tumors operated in 2002–2011

Table 1. Histological diagnosis of reoperated tumors

Carcinoma planoepitheliale	Carcinoma basocellulare	Carcinoma adenoidescysticum	Sarcoma	Carcinoma mucoepidermale	Carcinoma Solidum
168 (87.5%)	9 (4.7%)	6 (3.1%)	6 (3.1%)	2 (1.0%)	1 (0.5%)

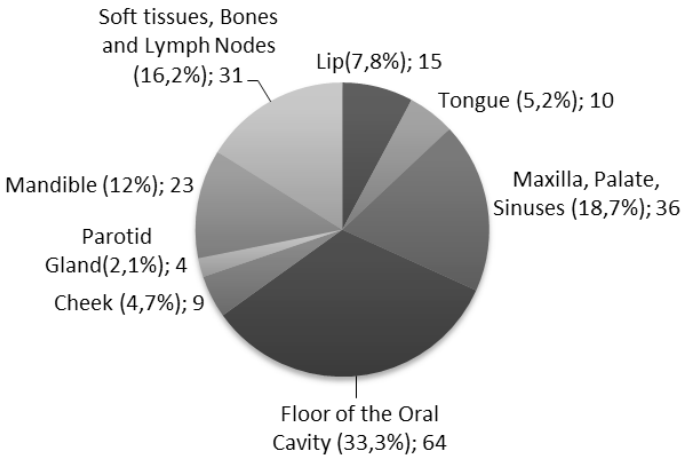

Figure 2. Number of surgical reoperations in specific primary locations of tumors

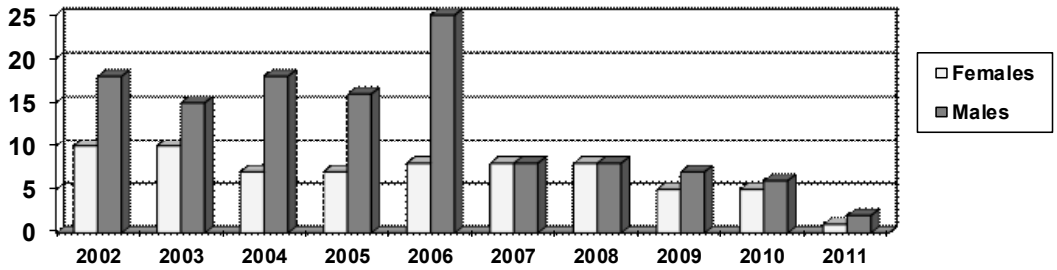

Figure 3. 192 patients treated due to malignant recurrences in 2002–2011 (69 females, 123 males)

Table 2. Age of reoperated patients

	under 40	41–50	51–60	61–70	71–80	over 80 years
Females	4	16	22	14	9	4
Males	13	21	53	19	12	5

recurrence, beginning with initial lesion to its removal, was about 15 months. Detailed description was presented in **Figure 4**.

Discussion

10 years of clinical observations provided us with a study group of 1697 patients treated for carcinoma. Annually, it averaged to 170 cases of oral and facial region. Comparison of data published in 1964–1994, by Flieger et al. [3], revealed 850 patients affected by malignancy (annually, 28 patients). Flieger's data revealed an increasing trend in the number of patients treated for carcinoma, confirming our hypothesis in terms of an increased number of carcinomas involving head and neck region. The peak of oncological involvement occured within the 5 and 6 decade with an apparent predominance of males [4]. Our results showed a similar pattern of age involvement.

According to the vast majority of literature, local and regional recurrences are observed within the first two years after initiation of treatment [1, 5–7]. Evaluation of our data has also showed this particular trend. Wolfensberg [8] reported loco regional recurrences in 18 out of 96 affected individuals within the three years after treatment initiation (18.7%). According to Petrisor and Fernandes [1] recurrences occur 2 years following treatment initiation, whereas Agra et al. [5] report 62.2% of recurrences, following the first year following treatment commencement. Recurrences within the lymph nodes were also reported within the same time frame by Kokenmuller et al. [6]. The data above indicates that the first two to three years following the surgical procedure, are a key determinant in diagnostic process of recurrences.

Recurrences were observed more frequently in individuals with a higher grade of malignancy G2-G3. This phenomenon was also observed by other authors [9, 10]. Location of the tumor and its recurrence in tumors of the mandible, tongue and buccal mucosa were statistically different among female group. According to Szybiak et al. [11], however, the frequency of tumor recur-

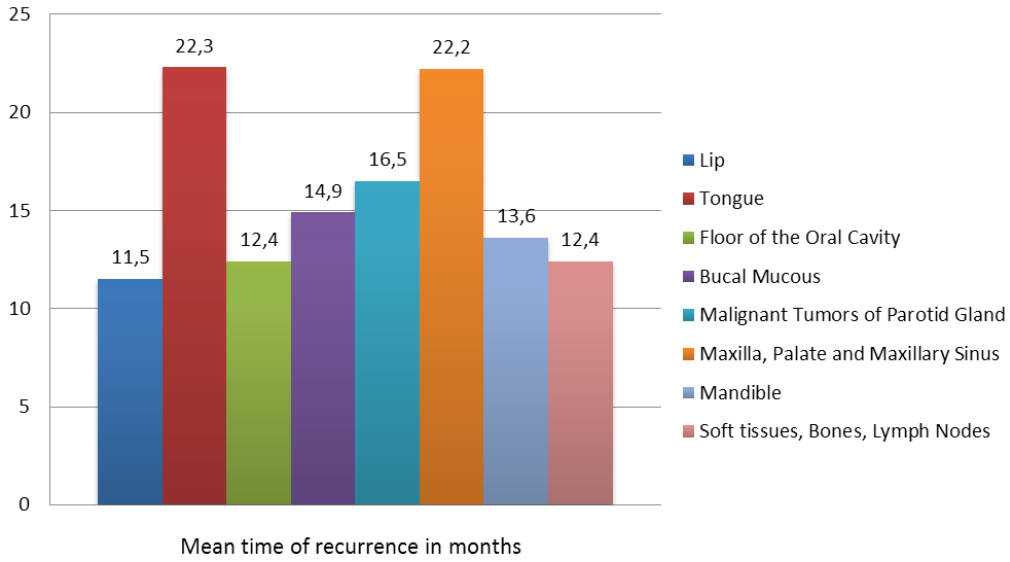

Figure 4. The average time of recurrence in specific primary locations of tumors

rence in the tongue region and floor of the mouth is 4 times higher in men than females. Our data correlates with the statistics of Szybiak et al. [11], who reported an increase of 16 percent recurrence rate. There is an apparent increase in frequency of tumor recurrence in the mandibular region and buccal mucosa in individuals with grade score G1.

There has also been reported a gender disproportionality, with a recurrence ratio of 55 to 9 in favor of the females, as well as a higher grade of pathological malignancy of the floor of the mouth tumor. According to our data, there has been a more frequent recurrence process within the region of the maxilla: 39.1%. (**Table 3**), without any gender difference, but with a higher grade of malignancy. The major reason for such high recurrence rate is a high grade of histological malignancy (G) as well as an anatomical complexity of the maxillary region, making a surgical intervention extremely challenging. Our data correlates with the literature and indicates a predominance of squamous cell carcinoma (87.5%) (**Table 1**). According to American research data [1], 90% of cancers affecting head, neck, and oral cavity region is squamous cell carcinoma.

Boysen et al. [12], on the other hand, stress that routine control after a three year time period following treatment initiation is rarely indicated. Boysen et al. [12], also does not recommend it in those in whom effective treatment methods have been exhausted. Visscher and Mani [13] claim that routine check-ups are crucial and emphasize that determination of location and grade of involvement are instrumental in determination of follow-up treatment duration. Hass et al. [14], emphasize the importance of routine check-ups in order to establish loco-regional recurrences, whenever there are feasible chances for effective treatment and of improvement of health. Early surgical intervention increases chances for successful treatment. According to the literature the clinical disease advancement has an instrumental influence on prognosis and survival of affected individuals. This has also been confirmed by Agra et al. [5].

In those in which recurrence has significantly advanced, as well as in those whom advanced primary

Table 3. Rate of recurrence after primary tumor operation in specific primary locations

Primary location	Rate of recurrence
Maxilla, Palate, Sinuses	39.1%
Cheek	19.1%
Lip	14.9%
Floor of the Oral Cavity	13.0%
Mandible	9.5%
Parotid Gland	8.5%
Tongue	6.6%
Soft tissues, Bones and Lymph Nodes	6.0%

lesion was previously treated surgically, the treatment outcomes are poor [15, 16]. Early diagnosis and immediate treatment commencement has greatly enhanced the tumor control rate within the head and neck region [8, 9, 17, 18].

Conclusions

Our data confirms the findings of other research groups in terms of accuracy of early diagnostic screening and its effect on final tumor treatment. It is also essential to accentuate the importance of post-treatment monitoring within first five years after initiation of treatment, which allows for an early determination of local recurrences and/or lymph node involvement.

Acknowledgements

Conflict of interest statement
The authors declare that there is no conflict of interest in the authorship or publication of contribution.

Funding sources
There are no sources of funding to declare.

References

1. Petrisor D, Fernandes R. Reoperative maxillofacial oncology, Oral Maxillofac Surg Clin North Am. 2011 Feb;23(1):161–168.
2. Nowotwory złośliwe w Wielkopolsce w 2009 roku. Biuletyn nr 8 Wydawnictwo Wielkopolskiego Centrum Onkologii w Poznaniu 2011; 9–19.
3. Flieger S, Lewandowski L, Osmola K, Nowaczyk MT. Leczenie chorych onkologicznych w klinice chirurgii Szczękowo-Twarzowej w Poznaniu w okresie 30 lat. Poznańska Stomatologia. 1994:13–17.
4. Lara PC, Bordón E, Rey A, Moreno M, Lloret M, Henríquez-Hernánde LA. IGF-1R expression predicts clinical outcome in patients with locally advanced oral squamous cell carcinoma. Oral Oncol. 2011 Jul;47(7):615–619.
5. Agra IM, Carvalho AL, Pinto CA, Martins EP, Filho JG, Soares FA et all. Biological markers and prognosis in recurrent oral cancer after salvage surgery. Arch Otolaryngol Head Neck Surg. 2008 Jul;134(7):743–749.
6. Kokemuller H, Brachvogel P, Eckardt A, Hausamen JE. Effectiveness of neck dissection in metastasizing mouth carcinoma. Uni- and multivariate analysis of factors of influence. Mund Kiefer Gesichtschir. 2002 Mar;6(2): 91–97.
7. Rowe DE, Carroll RJ, Day CL Jr. Prognostic factors for local recurrence, metastasis, and survival rates in squamous cell carcinoma of the skin, ear, and lip. Implications for treatment modality selection. J Am Acad Dermatol. 1992 Jun;26(6):976–990.
8. Wolfensberger M, Albrecht S, Muller W, Zbaren P, Dulguerov P, Arnoux A et all. Follow-up after histologically verified radical resection of early cancers of the mouth cavity: results of a prospective multicenter study. Schweiz Med Wochenschr Suppl. 2000;116:12S–14S.
9. Agra IM, Filho JG, Martins EP, Kowalski LP. Second salvage surgery for re-recurrent oral cavity and oropharynx carcinoma. Head Neck. 2010 Aug;32(8):997–1002.
10. Wong LY, Wei WI, Lam LK, Yuen AP. Salvage of recurrent head and neck squamous cell carcinoma after primary curative surgery. Head Neck. 2003 Nov;25(11):953–959.
11. Szybiak B, Trzeciak P, Golusiński W. Role of extended histological examination in the assessment of local recurrence of tongue and floor of the mouth cancer. Rep Pract Oncol Radiother. 2012 Nov;17(6):319–323.
12. Boysen M, Lövdal O, Tausjö J, Winther F. The value of follow-up in patients treated for squamous cell carcinoma of the head and neck. Eur J Cancer. 1992;28(2–3): 426–430.
13. de Visscher AV, Manni JJ. Routine long-term follow-up in patients treated with curative intent for squamous cell carcinoma of the larynx, pharynx, and oral cavity. Does it make sense? Arch Otolaryngol Head Neck Surg. 1994 Sep;120(9):934–939.
14. Haas I, Hauser U, Ganzer U. The dilemma of follow-up in head and neck cancer patients. Eur Arch Otorhinolaryngol. 2001 May;258(4):177–183.
15. Zafereo ME, Hanasono MM, Rosenthal DI, Sturgis EM, Lewin JS, Roberts DB et al. The role of salvage surgery in patients with recurrent squamous cell carcinoma of the oropharynx. Cancer. 2009 Dec;115(24):5723–5733.
16. Arnold DJ, Goodwin WJ, Weed DT, Civantos FJ. Treatment of recurrent and advanced stage squamous cell carcinoma of the head and neck. Semin Radiat Oncol. 2004 Apr;14(2):190–195.
17. Tsang RK, Chung JC, To VS, Chan JY, Ho WK, Wei WI. Efficacy of salvage neck dissection for isolated nodal recurrences in early carcinoma of oral tongue with watchful waiting management of initial N0 neck. Head Neck. 2011 Oct;33(10):1482–1485.
18. Schiefke F, Hildebrandt G, Pohlmann S, Heinicke F, Hemprich A, Frerich B. Combination of surgical resection and HDR brachytherapy in patients with recurrent or advanced head nad neck carcinomas. J Craniomaxillofac Surg. 2008 Jul;36(5):285–292.

Acceptance for editing: 2015-10-05
Acceptance for publication: 2015-12-17

Correspondence address:
Michał Marcinkowski
Department of Maxillofacial Surgery
Poznan University of Medical Sciences
49 Przybyszewskiego Street
60-355 Poznan, Poland
phone: +48 61 691402
fax: +48 61 8691687
email: michum1@wp.pl

ORIGINAL PAPER

DOI: https://doi.org/10.20883/medical.e11

Safety profile of anti-tumor necrosis factor therapy in inflammatory bowel disease – a single center experience

Piotr Eder, Liliana Łykowska-Szuber, Iwona Krela-Kaźmierczak, Kamila Stawczyk-Eder, Katarzyna Klimczak, Aleksandra Szymczak, Krzysztof Linke

Department of Gastroenterology, Human Nutrition and Internal Diseases, Poznan University of Medical Sciences
Heliodor Święcicki Clinical Hospital in Poznan, Poland

ABSTRACT

Introduction. The data on the safety profile of anti-tumor necrosis factor (anti-TNF) therapy in real-life patients cohorts with inflammatory bowel disease (IBD) still are lacking.
Aim. The aim of the study was to assess the adverse events (AE) of anti-TNF therapy in a single Polish IBD center.
Material and methods. Data on the safety of anti-TNF therapy among Crohn's disease (CD) and ulcerative colitis (UC) patients treated in years 2009–2015 at the Department of Gastroenterology, Human Nutrition and Internal Diseases of Poznań University of Medical Sciences were analyzed.
Results. There were 41 AE/188 therapies reported (21.8%) – 39/176 (22.1%) in CD and 2/12 (16.6%) in UC patients. The most common AE were infections – 10.1%, followed by dermatological complications – 2.6%, and infusion allergic reactions – 2.1%. The majority of AE (27/41 – 66%) were treated successfully or resolved without a treatment. The frequency of AE among patients treated with different molecules was similar – 17/85 (20%) in the adalimumab group, 20/91 (21.9%) in the originator infliximab group, and 4/18 (22.2%) in the biosimilar infliximab group. Concomitant treatment seemed not to influence the AE risk.
Conclusions. Anti-TNF therapy, which is used among the most severely ill IBD patients, seems to be safe. However, further assessment is needed in real-life patients cohorts, especially to assess the long-term safety of anti-TNF treatment in IBD.

Keywords: adalimumab, adverse events, Crohn's disease, infliximab, ulcerative colitis.

Introduction

Anti-tumor necrosis factor alpha (anti-TNF) therapy has significantly improved therapeutic possibilities in inflammatory bowel disease (IBD) in the last decade. High clinical efficacy of this novel therapy results in more and more common application of anti-TNF agents both in Crohn's disease (CD), and in ulcerative colitis (UC) [1]. Also in Poland, the number of anti-TNF-treated patients increases each year. Infliximab (IFX) and adalimumab (ADA) are the two most frequently used monoclonal antibodies neutralizing TNF-alpha in everyday clinical practice. Additionally, since 2014 new biosimilar forms of IFX are also used in Poland next to the originator IFX [2]. Since the introduction of biosimilars has decreased the costs of biological therapy, the treatment is becoming even more accessible.

One of the most important issues connected with each new therapy are safety profile and drug-related adverse events (AE). Anti-TNF therapy is generally considered to be safe, however, there are still many unanswered questions regarding, for example, the influence of IFX or ADA on the risk of infectious disease or malignancies [3].

The vast majority of data in terms of the safety profile of anti-TNF antibodies come from clinical trials. This has, however, significant limitations, as the populations included in clinical trials are strictly selected. Thus, the homogenous structure of the study groups in the trials does not reflect the heterogeneity and complexity of the clinical course of CD and UC. Moreover, each year new, unexpected and rare AE of anti-TNF treatment are reported. That is why we still need real-life data on the AE related to anti-TNF therapy in IBD. In the current study, we present our experience and data on the safety profile of anti-TNF treatment in IBD patients, treated in our center in years 2009–2015.

Material and methods

A retrospective analysis of the safety profile of anti-TNF therapy applied among IBD patients treated at the Department of Gastroenterology, Human Nutrition and Internal Diseases of Poznań University of Medical Sciences was performed. All AE reported from January 2009 till May 2015 were collected.

The AE was defined as each unexpected medical event occurring during or after stopping the anti-TNF therapy which might be connected with the treatment. The AE were divided into those which were undoubtedly related to anti-TNF therapy and in which relation to the treatment was questionable, but theoretically possible. We also divided them into AE occurring during the therapy and after stopping it.

Serious adverse event (SAE) was defined as each AE which significantly influenced the course of the therapy, resulting in death, a need for surgery or a need for the change in the concomitant pharmacological therapy, or a need for hospitalization.

Additionally, clinical data and laboratory results were collected.

An informed consent approved by the Institutional Review Board at the Heliodor Święcicki Clinical Hospital was obtained from each participant of the study.

Data were presented as means with standard deviations (SD). Categorized data were assessed with the Fisher exact test. A p value < 0.05 was considered significant. All data were analyzed using the GraphPad Prism Version 6.0 (GraphPad Software Inc., USA).

Results

12 UC and 134 CD patients were treated with anti-TNF antibodies between January 2009 and May 2015. Considering that, in the case of CD – 101 patients were treated once, 26 – twice, 5 patients – three times and 2 patients – four times, the whole number of inclusions to the biological therapy program was 188 (94 women and 94 men). The baseline characteristics of the analyzed group is presented in **Table 1**.

ADA was introduced 79 times, the originator IFX – 86 times and the biosimilar IFX 17 times. Additionally, in further 5 cases there was a need of changing the drug from the originator IFX to ADA and in 1 case

Table 1. Baseline characteristics of the whole study group (n = 188). Data are presented as means with standard deviations

Feature	Crohn's disease (n = 176)	Ulcerative colitis (n = 12)
Age (years)	34 ± 12	32 ± 12
Male/female – n	87/89	7/5
Disease duration (years)	5 ± 4	4 ± 3
C-reactive protein (mg/l)	26.6 ± 31.7	42.7 ± 31.6
Erythrocyte sedimentation rate (mm/h)	30 ± 21	43 ± 32
Hemoglobin (g/dl)	13.1 ± 6.1	11.6 ± 2.4
White blood cell count (10^3/mm^3)	7.8 ± 3.3	11.1 ± 3.1
Platelets (10^3/mm^3)	379 ± 130	363 ± 117
Disease location – %	L1 (ileal) – 38% L2 (colonic) – 20% L3 (ileocolonic) – 42%	E2 (left-sided) – 8% E3 (pancolitis) – 92%
Medications – %		
Steroids	44%	100%
Azathioprine	61%	66%
Aminosalicylates	90%	92%
Antibiotics	37%	100%

– from the biosimilar IFX to ADA, because of the injection allergic reaction.

There were 41 AE/188 therapies noted (frequency – 21.8%) – 39/176 (22.1%) in CD and 2/12 (16.6%) in UC patients. 25/41 (60.9%) were assessed as SAE [23/39 (58.9%) in CD, and 2/2 (100%) in UC patients]. In 7 cases the AE were undoubtedly related to anti-TNF therapy (4 cases of allergic infusion reaction, 2 cases of drug-induced psoriasis in the same patient, 1 case of skin abscess at a drug injection place). In 34 cases the association with anti-TNF treatment was only hypothetical and more or less probable. In 2 cases (colon lymphoma, perineal cancer) the adverse event occurred > 12 months after finishing the therapy, in 39 cases – during the therapy.

The characteristics of all AE in the study group is presented in **Table 2**.

27 AE were treated successfully or resolved without a treatment, in 8 cases – the therapy was not successful (4 recurrent skin infections, 2 cases of resistant drug-induced psoriasis, 1 case of recurrent Clostridium difficile infection, and 1 death because of cancer of perineal region). 5 patients are still under treatment because of the AE possibly related to anti-TNF therapy, in 1 case we have no data on the further course of the AE.

The frequency of AE among patients treated with different molecules was similar – 17/85 (20%) in the ADA exposed group, 20/91 (21.9%) in the originator IFX exposed patients, and 4/18 (22.2%) in the biosimilar IFX exposed group.

There were no statistically significant differences in the frequency of AE regarding the concomitant treatment with azathioprine (34% without azathioprine vs. 19% with azathioprine; p = 0.07), concomitant treatment with steroids (19% without steroids vs. 23.3% with steroids; p = 0.5). Patients who were treated for the second, third or fourth time were not at risk of developing more commonly AE when compared with those treated only once (26% vs. 20%, respectively; p = 0.5). The frequency of AE was higher among women, however, without statistical significance (24% vs. 19%, respectively; p = 0.5).

Discussion

Well balanced safety profile is one of the most important elements influencing the acceptance of every therapy. There are no medicines, however, which would not be associated with a risk of developing AE. The safety profile of anti-TNF antibodies seems to be acceptable, especially when considered their relevantly high clinical efficacy in untreatable conditions such as IBD [3, 4]. Nevertheless, there are still very few data in that subject coming from real-life patients populations. Moreover, there are no data describing this problem among adult IBD patients in Poland, and only one paper concerning Polish pediatric patients [5].

The overall rate of AE in our study cohort was 22%. This rate seems not to be high, when considered the qualification criteria for anti-TNF therapy in Poland.

Table 2. Characteristics of adverse events in patients with inflammatory bowel disease treated with anti-tumor necrosis factor therapy

	Crohn's disease (n = 176)	Ulcerative colitis (n = 12)	All patients (n = 188)
Infections	18	1	19 (10.1%)
Perianal abscess	6	0	6
Upper respiratory tract infections	3	0	3
Skin infections	8	0	8
Gastrointestinal infections	0	1	1
Other infections	1 case of varicella 1 intraabdominal abscess	0	2
Dermatological adverse events	2 cases of phototoxic reactions 2 cases of drug-induced psoriasis 1 case of dry skin syndrome	0	5 (2.6%)
Hematological adverse events	2 cases of leucopenia 1 case of thrombocytopenia	0	3 (1.5%)
Malignancies	1 case of perineal cancer 1 case of non-Hodgkin lymphoma	1 case of brain tumor	3 (1.5%)
Infusion allergic reactions	4	0	4 (2.1%)
Other adverse events	1 case of nausea 1 case of arthralgia 2 cases of liver enzymes elevation 2 cases of ileus	0	6 (3.1%)

Namely, we are using the "step-up" approach, in which only the most severely ill patients, not responding to all other classical drugs, can receive anti-TNF antibodies [6, 7].

It was shown, that the most frequent AE of anti-TNF therapy were infectious complications, which were noted among 10.1% of patients. This is in accordance with previous data [4]. For example, in a retrospective study on 500 patients treated with IFX in the Mayo Clinic, the frequency of infections was 8.2% [4, 8]. In a study by Lees et al., which included 620 patient-years of follow-up, the incidence of infections was 9.9% [4, 9]. The authors showed, that the majority of infectious AE occurred among patients receiving concomitant immunosuppressive drugs. In our patients' cohort we did not observe an increase of infections rate among patients on combo therapy. However, it has to be noted that the vast majority of patients were on steroids and/or azathioprine while starting anti-TNF treatment, so it is hard to definitively assess, whether this fact influenced the infectious complication risk. There were no cases of reactivated tuberculosis, which seems to be one of the most serious infectious AE in the course of anti-TNF therapy [10]. This shows that currently used algorithm for the exclusion of latent tuberculosis in Poland, using chest X-ray and skin tuberculin tests or interferon-gamma release assays (IGRA) before starting the treatment is effective.

One of the most frequent infectious complication in our study group were perianal abscesses (6 cases). This clinical situation could be also interpreted as a manifestation of CD, however, it seems that the link between anti-TNF therapy and the formation of perianal abscess is strong enough to classify it as AE [11]. This complication in all cases took place in the early phase of treatment and, hypothetically, was a result of closure of external skin opening of fistulas. This, in turn, prevented evacuation of infected content of the fistula tract which led to the collection of pus and formation of perianal abscess. Since this is a severe complication, we changed the therapeutic approach in the cases of complex perianal CD, performing parallel aggressive surgical treatment, including negative wound pressure therapy. As a result, since 2013, there has been only one case of perianal abscess. This example shows that the optimization and individualization of anti-TNF therapy can improve therapeutic outcomes and decrease the risk of AE.

Interestingly, we noted also several cases of dermatological AE in our series. This number could be even greater if injection site reactions or skin infections were classified to this subgroup of AE. This is in accordance with the current knowledge in terms of the safety of anti-TNF agents [12, 13]. It seems that dermatological effects of anti-TNF treatment were underestimated. Rheumatologic data show that even up to 25% of patients on anti-TNF drugs had dermatological complications, and this was also confirmed in IBD patients [13, 14]. The most clinically significant dermatological AE seem to be skin malignancies. There are, however, conflicting data in terms of the influence of anti-TNF therapy on the risk of non-melanoma skin cancer (NMSC) or skin melanoma [13]. Moreover, in a study performed by Long et al. in a cohort of 108579 IBD patients it was shown that the disease by itself can be associated with higher risk of skin melanoma and NMSC, especially in individuals with CD [15]. Additionally, immunosuppressive drugs, like azathioprine and methotrexate, can also influence the malignancy rates [13].

In our study cohort there was no case of skin malignancy, but we noted another interesting dermatological AE – drug-induced psoriasis. This was observed twice in the same CD patient (the first time – during the course of maintenance IFX treatment, and then three years later – as a result of ADA therapy). The etiology of this immune-mediated complication is unknown [13]. It is believed that anti-TNF agents, being anti-inflammatory molecules, in some proportion of patients can cause paradoxical pro-inflammatory reactions, which is hypothetically mediated by a local uncontrolled production of interferon-α, resulting from TNF blockade [13, 16]. Current data show, that this phenomenon is not rare. In one of the most recent reviews of this subject, Denadai et al. reported that the highest risk of developing anti-TNF-induced psoriasis is among CD patients on IFX [17]. Skin lesions can appear at any moment of the therapy, but the most frequent time is between third and fourth IFX infusion [13].

We reported 3 cases of malignancies in our study group. In the case of two of them, however, the link between anti-TNF therapy was very weak. In the case of a patient with perineal cancer, the main etiological factor was a severe, long-lasting perianal CD, with multiple perianal and vaginal fistulas. In the second one – brain tumor was diagnosed just after first dose of IFX in a patient with fulminant UC, so the association with anti-TNF therapy is very unlikely. We also reported a case of non-Hodgkin lymphoma (NHL) of the colon. This patient was treated with ADA, however, the patient was also treated with experimental drug in clinical trials in the past because of a very severe CD course. That is why, it is also difficult to assess what was the main etiological factor of NHL in this case – anti-TNF thera-

py, concomitant and past treatment, or active, severe and long-lasting disease by itself. The literature review also brings conflicting data in terms of the association between anti-TNF therapy and lymphoma risk [10, 18, 19]. Recent meta-analyses show that the risk of developing lymphoma is slightly increased in patients with IBD and it is related to the use of immunosuppressive drugs or both – immunosuppressants together with anti-TNF agents [18].

Our study has several limitations. The main one is the retrospective nature of the analysis, as it theoretically could lead to underestimation of the AE rate. However, as it was also noted in another retrospective study on the safety of anti-TNF therapy among Polish pediatric IBD patients, all patients included in the analysis were treated according to the guidelines of Polish National Health Fund, and we are obliged to strictly monitor the course of the therapy [5]. As a result, all data are registered and collected, thus they seem to be reliable enough to perform the analysis. Another limitation is the definition of AE, as it may influence the rate of reported complications of the therapy. Some of the were AE reported, however, their association with anti-TNF therapy seemed to be very poor. On the other hand, we excluded some clinical situations (for example anemia), recognizing them as disease manifestation, not therapeutic complications. Nevertheless, it should be mentioned, that there is no commonly accepted definition of AE, and all data in this subject should be interpreted with respect to specific definition used in a particular, analyzed study.

Conclusions

To conclude, in the current study safety profile of anti-TNF therapy in IBD real-life patients cohort is presented. It was shown for the first time among Polish adult IBD patients, that the therapy is generally well-tolerated and the majority of the AE disappear after stopping the treatment and/or introducing a specific therapy. We did not show any significant differences in drug-related complication rate either between different molecules or between the originator and biosimilar IFX, however, the number of patients treated with biosimilars is still small. Proper qualification to the therapy, strict monitoring and optimizing the treatment can decrease or eliminate the risk of several AE. However, since the observation time of the safety profile of anti-TNF treatment is still short (10–15 years), further assessment is needed in this respect, especially in terms of long-term therapy of IBD.

Acknowledgements

We would like to thank Agnieszka Kurek for her writing assistance.

Conflict of interest statement

The authors declare that there is no conflict of interest in the authorship or publication of contribution.

Funding sources

P. Eder, L. Łykowska-Szuber, K. Klimczak received lecture fees from Abbvie Poland.

References

1. Amiot A, Peyri-Biroulet L. Current, new and future biological agents on the horizon for the treatment of inflammatory bowel diseases. Therap Adv Gastroenterol. 2015 Mar;8(2):66–82.
2. Gomollon F. Biosimilars in inflammatory bowel disease: ready for prime time? Curr Opin Gastroenterol. 2015 Jul;31(4):290–295.
3. Sousa P, Allez M. Complications of biologics in inflammatory bowel disease. Curr Opin Gastroenterol. 2015 Jul;31(4):296–302.
4. Stallmach A, Hagel S, Bruns T. Adverse effects of biologics used for treating IBD. Best Pract Res Clin Gastroenterol. 2010 Apr;24(2):167–182.
5. Szymańska E, Dądalski M, Oracz G, Kierkuś J. Safety profile of anti-TNF agents in Polish pediatric patients with Crohn's disease. Austin J Gastroenterol. 2014 Aug;1(4): 1016.
6. Łodyga M, Eder P, Bartnik W, Gonciarz M, Kłopocka M, Linke K et al. Guidelines for the management of Crohn's disease. Recommendations of the Working Group of the Polish National Consultant in gastroenterology and the Polish Society of Gastroenterology. Prz Gastroenterol 2012 Dec;7(6):317–338.
7. Eder P, Łodyga M, Łykowska-Szuber L, Bartnik W, Durlik M, Gonciarz M et al. Guidelines for the management of ulcerative colitis. Recommendations of the Working Group of the Polish National Consultant in gastroenterology and the Polish Society of Gastroenterology. Prz Gastroenterol 2013 Jan;8(1):1–20.
8. Colombel JF, Loftus EV, Tremaine WJ, Egan EJ, Harmsen WS, Schleck CD et al. The safety profile of infliximab in patients with Crohn's disease: the Mayo Clinic experience in 500 patients. Gastroenterology. 2004 Jan;126(1): 19–31.
9. Lees CW, Ali AI, Thompson AI, Ho GT, Forsythe RO, Marquez L et al. The safety profile of anti-tumour necrosis factor therapy in inflammatory bowel disease in clinical practice: analysis of 620 patient-years follow-up. Aliment Pharmacol Ther. 2009 Feb;29(3):286–297.
10. Targownik LE, Bernstein CN. Infectious and malignant complications of TNF inhibitor therapy in IBD. Am J Gastroenterol. 2013 Dec;108(12):1835–1842.
11. Gecse K, Khanna R, Stoker J, Jenkins JT, Gabe S, Hahnloser D et al. Fistulizing Crohn's disease: Diagnosis and management. United European Gastroenterol J. 2013 Jun;1(3):206–213.
12. Freling E, Baumann C, Cuny JF, Bigard MA, Schmutz JL, Barbaud A et al. Cumulative incidence of, risk factors for, and outcome of dermatological complications of anti-

-TNF therapy in inflammatory bowel disease: a 14-year experience. Am J Gastroenterol. 2015 Aug;110(8):1186–1196.
13. Mocci G, Marzo M, Papa A, Armuzzi A, Guidi L. Dermatological adverse reactions during anti-TNF treatments: focus on inflammatory bowel disease. J Crohns Colitis. 2013 Nov;7(10):769–779.
14. Lee HH, Song IH, Friedrich M, Gauliard A, Detert J, Rowert J et al. Cutaneous side-effects in patients with rheumatic diseases during application of tumour necrosis factor-alpha antagonists. Br J Dermatol. 2007 Mar;156(3):486–491.
15. Long MD, Martin CF, Pipkin CA, Herfarth HH, Sandler RS, Kappelman MD. Risk of melanoma and nonmelanoma skin cancer among patients with inflammatory bowel disease. Gastroenterology. 2012 Aug;143(2):390–399.
16. Cullen G, Kroshinsky D, Cheifetz AS, Korzenik JR. Psoriasis associated with anti-tumour necrosis factor therapy in inflammatory bowel disease: a new series and a review of 120 cases from the literature. Aliment Pharmacol Ther. 2011 Dec;34(11–12):1318–1327.
17. Denadai R, Teixera FV, Saad-Hossne R. The onset of psoriasis during the treatment of inflammatory bowel diseases with infliximab: should biological therapy be suspended? Arq Gastroenterol. 2012 Apr-Jun;49(2):172–176.
18. Lakatos PL, Miheller P. Is there an increased risk of lymphoma and malignancies under anti-TNF therapy in IBD? Curr Drug targets. 2010 Feb;11(2):179–186.
19. Kopylov U, Vutcovici M, Kezouh A, Seidman E, Bitton A, Afif W. Risk of lymphoma, colorectal and skin cancer in patients with IBD treated with immunomodulators and biologics: a Quebec claims database study. Inflamm Bowel Dis. 2015 Aug;21(8):1847–1853.

Acceptance for editing: 2015-02-10
Acceptance for publication: 2015-03-30

Correspondence address:
Piotr Eder
Department of Gastroenterology, Human Nutrition and Internal Diseases, Poznan University of Medical Sciences
Heliodor Swiecicki Clinical Hospital
49 Przybyszewskiego Street, 60-355 Poznan, Poland
phone: +48 61 8691343
fax: +48 61 8691686
email: piotr.eder@op.pl

ORIGINAL PAPER

DOI: https://doi.org/10.20883/medical.e12

Retrospective analysis of infections prevalence in patients with progressive systemic sclerosis treated with cyclophosphamide

Małgorzata Schlabs[1, 2], Katarzyna Pawlak-Buś[1, 2], Piotr Leszczyński[1, 2]

[1] Department of Rheumatology and Osteoporosis, Jozef Strus Municipal Hospital in Poznan, Poland
[2] Department of Rheumatology and Rehabilitation, Poznan University of Medical Sciences, Poland

ABSTRACT

Aim. Assessment of infections prevalence rate, type and severity in patients diagnosed with progressive systemic sclerosis (PSS), treated with cyclophosphamide (CTX), during 12 months of observations.
Material and methods. A retrospective analysis of mild, moderate and severe infections in 17 women with a mean age of 58.8 ± 10.0, based on an interview, physical examination, additional tests, and available medical records.
Results. 46 various infections were diagnosed in the analysed group of patients. 32 (69.6%) infections involved the respiratory system, and 14 (30.4%) infections concerned the urinary tract. The average frequency per one patient was 2.7 ± 3.5 (median: 2) events during 12 months of observations. The majority of infections, 60.9 % (n = 28), were mild ones of slight intensity, and 37.0% (n = 17) were moderate ones. Only one person (2.2% of all infections) had a severe infection requiring hospitalisation.
Conclusions. In the studied group the infection prevalence rate was comparable to that in a healthy population. The majority of infections were mild and involved the respiratory system. Basis of conducted analysis 12 months intravenous administration of CTX is not a factor significantly increasing a risk of severe infections in the studied group of patients. In PSS patients CTX pulse therapy is relatively safe, as it does not cause severe infections requiring hospitalisation.

Keywords: infections, progressive systemic sclerosis, cyclophosphamide treatment.

Introduction

Progressive systemic sclerosis (PSS) is one of the most severe systemic diseases of connective tissue. Causal treatment of PSS is yet unknown. Currently used therapies only aim at inhibiting the inflammatory process and progressive multi-organ fibrosis. One of the treatment methods known for years is immunosuppression with cyclophosphamide (CTX). This medicine belongs to standard cytostatic agents used in treatment of autoimmune diseases and cancers. The anti-inflammatory mechanism underlying the effect of CTX metabolites is based on alkylation of deoxyribonucleic acid (DNA) in mature T- and B-cells (and, to a smaller extent, bone marrow precursor cells), resulting in their damage and death [1, 2]. In PSS cyclophosphamide inhibits progression of interstitial lesions in lungs and has an advantageous influence on skin sclerosis remission [2–4]. It also improves respiratory system efficiency measured as forced vital capacity (FVC) [3, 4, 6], total lung capacity (TLC) [4] and *diffusing* capacity of the lungs for carbon monoxide (DLCO) [7, 8]. Therefore CTX treatment improves quality of patients' life [3]. Recent recommendations of The European League Against Rheumatism (EULAR), published in 2009, recommend considering use of CTX for early lung lesions, scleroderma-related interstitial lung disease (SSc-ILD), as remission-inducing therapy [10]. Despite proven

efficacy and safety of CTX, this treatment may involve some adverse effects, including infections [3, 10]. In studies on using CTX in patients with PSS and other autoimmune diseases (vasculitis, systemic lupus erythematosus and others) it was observed that benefits of this therapy exceed a related risk of possible infections [11]. Infection severity depends mainly on the administration route and a cumulative dose of CTX. High-dose intravenous CTX therapy (50 mg/kg/d for 4 consecutive days) not related to a stem cells transplant is mainly applied in treatment of aplastic anaemia [2, 9]. This treatment was also attempted in PSS patients in the past, and it resulted in skin lesions remission and improvement in quality of life [9, 12]. However, this regimen and dosing is associated with a significantly higher number of complications and sometimes required numerous preventive activities, including administration of the *granulocyte* colony-stimulating factor (G-*CSF*), mesna, antibiotic therapy, or antifungal treatment. Therefore, currently this regimen is not recommended for PSS treatment. Oral CTX administration at 1–2 mg/kg/daily results in an even distribution of the dose, usually not requiring prevention of severe infections; however, it is still related to a high cumulative dose, increasing the rate of adverse effects [3, 6]. However, this dose can be significantly reduced and yet as effective, when CTX is administered intravenously in intravenous pulse therapy at low single doses. Using results of a multicentre, prospective, randomised, double-blind study, it is recommended to replace the oral drug with CTX at a dose of 600 mg/m², intravenously, once a month for six months [5]. There are numerous examples in the literature confirming effectiveness of low-dose intravenous CTX therapy in PSS patients [11, 13, 14, 15]. This treatment regimen may reduce a risk of various adverse effects, including infections, during long-term therapy.

Material and methods

All 17 women who were included in this study fulfilled the PSS classification criteria of The American College of Rheumatology / The European League Against Rheumatism (ACR/EULAR 2013) [15]. Selected population of patients, received CTX intravenously in a pulse therapy during the mean time of 12.0 ± 4.0 months. The mean age in the studied group was 58.8 ± 10.0 years (median: 60 years), and the mean disease duration from the moment of its diagnosis was 53.7 ± 51.1 months (median: 36 months). The patients were treated with CTX at a dose of 400–800 mg, applied as a single dose every 1–3 months. In the studied group, the mean CTX cumulative dose was 9870.6 mg ± 6724.2 mg (median: 8600 mg), ranging from 2000–25600 mg (**Table 1**).

Table 1. Characteristic of patients with PSS

N	Age (years)	DD (months)	CTX (mg)	ANA[1]	Immunological profile[2]			Pulmonary changes[3]	PAH[4]
					Scl 70	CENB	PM-Scl		
1.	64	108	22800	positive	+++	–	–	(+++)	yes
2.	49	84	25600	positive	+++	–	–	(+++)	yes
3.	54	12	4200	positive	+++	–	–	negative	–
4.	65	36	10800	positive	+++	–	–	(+)	–
5.	61	132	10000	positive	–	–	–	(+++)	yes
6.	57	60	10200	positive	+	+++	–	(+++)	–
7.	52	193	5200	positive	–	–	–	(++)	–
8.	47	24	11000	positive	+	–	–	(++)	yes
9.	62	24	7000	positive	–	–	–	(+++)	yes
10.	62	12	6000	positive	+++	–	–	(++)	–
11.	36	24	9000	positive	–	+	+++	(++)	yes
12.	66	12	6000	positive	–	–	–	(+)	–
13.	60	60	8600	positive	–	+++	–	(+++)	–
14.	59	12	4600	positive	–	–	–	(+++)	–
15.	76	36	5000	positive	+	–	–	(+++)	yes
16.	54	12	2000	–	–	–	–	(+++)	–
17.	76	72	19800	positive	+++	–	–	(+++)	–

1. Detected by the immunoenzymatic test ANA Screen; laboratory standard: < 40 U/ml
2. + – weakly positive result; ++ – positive result; +++ – strong positive result
3. On a basis of computed tomography; (+) – minimal, preliminary changes, basal fibrosis; (++) – interstitial changes; (+++) – ground-glass opacities, honeycombing
4. Pulmonary arterial hypertension is probable: RVSP > 30mmHg in the transthoracic echocardiography

N – the number of patients; DD – diseases duration; ANA – antinuclear antibodies; Scl 70 – anti- topomisomerase I antibodies; CENB – anti-centromere protein B antibodies; PM-Scl – anti- polymyositis/scleroderma antibodies; PAH – pulmonary arterial hypertension

Table 2. Classifications of severity and type of the studied infections in patients with PSS

Type of infection	Treatment of infection	Localization of infection
Mild infection	Infection of mild intensity treated without antibiotics, antiviral or antifungal medications	the upper respiratory tract
		the urinary tract
		skin and/or subcutaneous tissue
Moderate infection	Infection treated with antibiotics, antiviral or antifungal medications in the ambulatory care, without complications, with rapid regression of the symptoms	the upper respiratory tract
		the lower respiratory tract
		the urinary tract
		skin and/or subcutaneous tissue
Severe infection	Infection requiring hospitalisation, treated by intravenous drugs or recurrent, chronic, resistant to standard treatment	the upper respiratory tract
		the lower respiratory tract
		the urinary tract
		skin and/or subcutaneous tissue

Table 3. Infections prevalence rate, type and severity in studied group of patients with Progressive Systemic Sclerosis

N	Mild infection			Moderate infection				Severe infection			
	URT	UT	S/SCT	URT	LRT	UT	S/SCT	URT	LRT	UT	S/SCT
1.	–	–	–	–	–	–	–	–	–	–	–
2.	1	–	–	1	–	–	–	–	–	–	–
3.	–	1	–	–	–	1	–	–	–	–	–
4.	2	–	–	–	–	–	–	–	–	–	–
5.	–	–	–	1	–	–	–	–	–	–	–
6.	–	–	–	3	–	–	–	–	–	–	–
7.	5	3	–	–	2	–	–	–	1	–	–
8.	2	–	–	–	–	–	–	–	–	–	–
9.	–	1	–	–	–	–	–	–	–	–	–
10.	–	–	–	–	–	–	–	–	–	–	–
11.	4	–	–	–	–	–	–	–	–	–	–
12.	2	–	–	–	–	–	–	–	–	–	–
13.	–	–	–	–	1	–	–	–	–	–	–
14.	–	–	–	1	–	–	–	–	–	–	–
15.	2	5	–	3	1	1	–	–	–	–	–
16.	–	–	–	–	–	–	–	–	–	–	–
17.	–	–	–	–	–	2	–	–	–	–	–

N – the number of patients; URT – the upper respiratory tract; LRT – the lower respiratory tract; UT – the urinary tract; S/SCT – skin and/or subcutaneous tissue

A retrospective 12-month analysis of infections was conducted in the studied group of PSS patients receiving long-term pulse CTX treatment. The analysis used data collected during interviews, physical examinations, and additional tests, together with all available medical records. For the needs of the study, the infections were divided into three categories: type, treatment options, localization, and also criteria for their classification according to their severity were established (**Table 2**). In this study the arithmetic mean of infections in each patient was calculated.

Results

In the studied group of patients with PSS treated with CTX pulse therapy, 46 various infections were found in total during the year analysed retrospectively. The following infection types were found: 28 (60.9%) mild infections, 17 (37.0%) moderate infections, and a severe infection in one (2.2%) patient. Only 4 (23.5%) had more than 2 infections a year. In each patient, the mean number of infections in the analysed year was 2.7 ± 3.5 (median: 2). The majority (32 – 69.6%) of infections concerned the respira-

tory system, while the remaining 14 (30.4%) affected the urinary tract. No skin and/or subcutaneous tissue infections were found in the analysed group of patients. Only in one case the infection required hospitalisation and parenteral antibiotic therapy. In three (17.7%) patients no infection was diagnosed in the period covered by observations.

Discussion

In the conducted retrospective analysis of the group of PSS patients treated with intravenously administered CTX, the majority of infections were mild and transient. The patients covered by the assessment presented mainly the respiratory system infections, of which over 80% involved the upper respiratory tract. They were mild and required only a symptomatic treatment. In a population of healthy adults, 2–5 cases of viral acute rhino-sinusitis, including common cold, were noted per year, and this is comparable to the rate of majority of infections in the studied group [17]. Our analysis showed that patients receiving CTX as a long pulse therapy of single 400–800 mg doses every 1–3 month do not develop a significant infection frequently, and severity of most of them possibly does not outbalance scientifically proven advantages of this treatment. Basing on the studied group CTX is not a significant factor increasing a risk of severe infections requiring parenteral treatment and/or hospital admission.

CTX therapy inhibits the immune system; therefore, by definition it should increase frequency and severity of infections. Considering the conducted study, it can be established that it is not so, particularly in a case of standard immunosuppressive PSS treatment. In the analysed group of patients, more than half of infections were mild, not requiring causal treatment, and nearly 20% of patients did not have any infection in the analysed period of time. Only one person suffered a severe infection in form of bronchitis, but it was without complications and resolved with intravenous empiric antibiotic therapy. The conducted analysis was certainly limited by a lack of a relevant control group of PSS patients not receiving immunosuppressive treatment, as well as by inability to obtain details on infection aetiology. Also, it is difficult to compare the results of this study with other reports, as publications on infections in PSS patients chronically treated with CTX are lacking. Nevertheless, the analysis showed that pulse CTX therapy does not increase frequency of severe infections in PSS patients.

Acknowledgements

Conflict of interest statement
The authors declare that there is no conflict of interest in the authorship or publication of contribution.

Funding sources
There are no sources of funding to declare.

References

1. Manno R, Boin F. Immunotherapy of systemic sclerosis. Immunotherapy. 2010 Nov;2(6):863–878.
2. DeZern AE, Petri M, Drahman DB et al. High dose cyclophosphamide without stem cell rescue in 207 patients with aplastic anemia and other autoimmune diseases.
3. Tashkin DP, Elashoff R, Clements PJ et al. Scleroderma Lung Study Research Group. Cyclophosphamide versus placebo in scleroderma lung disease. N Engl J Med. 2006 Jun 22;354(25):2655–66.
4. Akesson A, Scheja A, Lundin A et al. Improved pulmonary function in systemic sclerosis after treatment with cyclophosphamide. Arthritis Rheum. 1994 May;37(5): 729–35.
5. Hoyles RK, Ellis RW, Wellsbury J et al. A multicenter, prospective, randomized, double-blind, placebo-controlled trial of corticosteroids and intravenous cyclophosphamide followed by oral azathioprine for the treatment of pulmonary fibrosis in scleroderma. Arthritis Rheum. 2006;54:3962–70.
6. Silver RM, Warrick JH, Kinsella MB et al. Cyclophosphamide and low-dose prednisone therapy in patients with systemic sclerosis (scleroderma) with interstitial lung disease. J Rheumatol. 1993 May;20(5):838–44.
7. Davas EM, Peppas C, Maragou M et al. Intravenous cyclophosphamide pulse therapy for the treatment of lung disease associated with scleroderma. Clin Rheumatol. 1999;18(6):455–61.
8. White B, Moore WC, Wigley FM et al. Cyclophosphamide is associated with pulmonary function and survival benefit in patients with scleroderma and alveolitis. Ann Intern Med. 2000 Jun 20;132(12):947–54.
9. Dezern AE, Styler MJ, Drachman DB et al. Repeated treatment with high dose cyclophosphamide for severe autoimmune diseases. Am J. Blood Res. 2013;3(1):84–90.
10. Kowal-Bielecka O, Landewé R, Avouac J et al. EULAR recommendations for the treatment of systemic sclerosis: a report from the EULAR Scleroderma Trials and Research group (EUSTAR). Ann Rheum Dis. 2009 May;68(5):620–8.
11. Haga HJ, D'Cruz D, Asherson R et al. Short term effects of intravenouspulses of cyclophosphamide in the treatment of connective tissue disease crisis. Ann Rheum Dis. 1992 Jul;51(7):885–8.
12. Tehlirian CV, Hummers LK, White B et al. High-dose cyclophosphamide without stem cell rescue in scleroderma. Ann Rheum Dis. 2008 Jun;67(6):775–81.
13. D'Angelo S, Cuomo G, Paone C et al. Low-dose intravenous cyclophosphamide in systemic sclerosis: a preliminary safety study. Clin Rheumatol. 2003 Dec;22(6):393–6.
14. Tochimoto A, Kawaguchi Y, Hara M et al. Efficacy and safety of intravenous cyclophosphamide pulse therapy with oral prednisolone in the treatment of intersti-

tial lung disease with systemic sclerosis: 4-year follow-up. Mod Rheumatol. 2011 Jun;21(3):296–301.
15. Várai G, Earle L, Jimenez SA et al. A pilot study of intermittent intravenous cyclophosphamide for the treatment of systemic sclerosis associated lung disease. J Rheumatol. 1998 Jul;25(7):1325–9.
16. Van den Hoogen F, Khanna D, Fransen J et al. 2013 classification criteria for systemic sclerosis: an American college of rheumatology/European league against rheumatism collaborative initiative. Ann Rheum Dis. 2013 Nov;72(11):1747–55.
17. Fokkens WJ, Lund VJ, Mullol J et al. European Position on Rhinosinusitis and Nasal Polyps 2012. Epidemiology of ARS. Rhinology, 2012; suppl. 23:9–16.

Acceptance for editing: 2015-02-10
Acceptance for publication: 2015-03-30

Correspondence address:
Małgorzata Schlabs
Piotr Leszczyński
Department of Rheumatology and Osteoporosis
Jozef Strus Hospital
61-285 Poznań, Szwajcarska 3
Secretariat: phone: +48 61 8739260
fax: +48 61 8739260
email: gosia.schlabs@op.pl, piotr_leszczynski@wp.pl

ORIGINAL PAPER

DOI: https://doi.org/10.20883/medical.e13

Early differentation of lamellar structure of the intervertebral disc in staged human embryos

Witold Woźniak, Małgorzata Grzymisławska, Joanna Łupicka, Małgorzata Bruska, Adam Piotrowski, Anna Gałązka, Magdalena Rojewska, Jarosław Sobański

Department of Anatomy, Poznan University of Medical Sciences, Poland

ABSTRACT

Introduction. In the vast literature concerning the development of the intervertebral discs controversies exist as to the period of differentiation and structure of the nucleus pulposus and annulus fibrosus. These controversies result from different determination of age of the investigated embryos.
Aim. Using embryos from departmental collection age of which was established according to international Carnegie staging and expressed in postfertilizational days, the differentiation of the intervertebral discs was traced.
Material and methods. Study was performed on 34 embryos at developmental stages 13–23 (32–56 days). Embryos were serially sectioned in sagittal, frontal and horizontal planes. Sections were stained with various histological methods and impregnated with silver.
Results. Division of sclerotomes into loose cranial and dense caudal zones (sclerotomites) was observed in embryos aged 32 days (stage 13). The intervertebral disc developed from the dense zone of sclerotome and was well recognized in embryos aged 33 days (stage 14). At the end of fifth week (embryos at stage 15, 36 days) the annulus fibrosus and the nucleus pulposus were seen. The annulus fibrosus differentiated into lateral and medial zones. Within the lateral zone cells were arranged into circular rows. These rows were considered as the first stage of laminar structure. In further developmental stages the laminae occupied both zones of the annulus fibrosus.
Conclusions. The intervertebral discs develop from the dense zone of the sclerotome which is evident in embryos at stage 13 (32 days). Discs differentiate in embryos aged 33 days, when the nucleus pulposus and annulus fibrosus are recognized. In embryos aged 36 days in the annulus fibrosus circular rows forming laminar arrangement are seen.

Keywords: human embryology, intervertebral disc, annulus fibrosus.

Introduction

The intervertebral disc is a moderately movable joint that separates the vertebrae of the vertebral column [1]. The disc was first recognized as an anatomical entity by Vesalius and together with its subjacent vertebral bodies and associated ligaments it constitutes the vertebral unit [2].

The adult intervertebral disc is an avascular fibrocartilage tissue with a small population of cells that obtain nutrients from the capillaries at the vertebral-disc body interface [3].

Nutrients diffuse from capillaries to the disc cells and metabolities produced then diffuse from the cells through the matrix to the blood supply [4].

The disc is composed of a nucleus pulposus, which occupies the center of the disc, and an outer zone, annulus fibrosus consisting of a series of lamellae of collagenous bundles, which are arranged spirally.

The disc vertebral-interface of cartilage endplate is considered by some authors as a separate third structure of the intervertebral disc [5, 6].

Each of these tissues has a different function and consists of a specific matrix structure that is maintained by distinct cell populations [1].

The nucleus pulposus is hydrated semigelatinous tissue which contains fine bundles of collagenous fibers, type II connective tissue cells, and chondrocyte-like cells, embedded in amorphous intercellular matrix containing hydrophilic proteoglycans which retain water and provide strength and spongy features to the disc [2]. It is predominantly made up of water (70–90% depending on the age). Collagenous fibers constite ca. 20% of dry weight and proteoglycans ca. 50% of dry weight [7]. However, tissue of nucleus pulposus is different from hyaline cartilage due to the ratio between proteoglycan and collagen. This ratio within the nucleus pulposus is 27:1, whereas in cartilaginous endplate it is 2:1 [1, 8].

The annulus fibrosus is organized in fibrous ring-like lamellae and it surrounds the nucleus pulposus. It may be divided into inner and outer zones and is attached to the vertebrae at the superior and inferior surfaces as well as is also connected to anterior and posterior longitudinal ligaments. The thickness of the lamellae increases from the outer to inner layers [9].

The annulus fibrosus is composed principally of water (60–80%, depending on the region and age), collagen (50–70% of dry weight), aggregating and nonaggregating proteoglycans (10–20% of dry weight) and noncollagenous proteins (ca. 25% of dry weight) [1].

Within each lamella collagen fibers are lying parallel [9]. In addition to the collagen network, a network of elastin fibers is present between the lamellae [10].

The cartilaginous endplate is composed of an osseous and a cartilaginous part. The main component of the endplate is water (80% after birth, and 70% after 15 years age), followed by type II collagenous fibers and proteoglycans [1]. The thickness of the human endplate is 0.5–1 mm at the periphery and diminishes toward the center. It has a semi-permeable barrier and load-bearing functions [1].

The development of the intervertebral disc in human has been investigated by many authors [11–15].

There are many controversies in literature as to the contribution of loose and dense zones of sclerotomes and perinotochordal sheath to the development of the intervertebral disc. Different opinions also exist considering early differentiation of the annulus fibrosus and nucleus pulposus. It seems not amiss, therefore, to present the development of the intervertebral disc in staged human embryos with particular consideration of the annulus fibrosus.

Knowledge of the embryonic development of the intervertebral disc is important in understanding the adult regeneration of the disc. Such developmental studies are also needed to elucidate problems of chronic back pains connected with disc degeneration.

Material and methods

Study was made on 34 serially sectioned human embryos of developmental stages 13–23 from the Collection of the Department of Anatomy, Poznan University of Medical Sciences. Age of embryos was established according to 23 international stages and was expressed in postfertilizational days (**Table 1**). Embryos were cut in sagittal, frontal and horizontal planes.

Serial sections of 5 or 10 micrometers thickness were stained according to following methods: 1) hematoxylin and eosin, 2) Nissl's toluidine blue or cresyl violet, 3) Mallory's trichrome, 4) Bodian's protargol.

In each developmental stage the graphic reconstructions, based on sections, were made.

Results

In embryos at stage 13 (32 days) the notochord is a continuous, unconstructed rod and forms the central axis for the developing vertebral column. The number of somites is 36 and they differentiate into sclerotomes, myotomes and dermatomes. The division of sclerotomes into loose, cranial and dense, caudal zones commences. These zones are also called sclerotomites. Intrasclerotomic fissures are distinctly seen and the loose cranial sclerotomite is traversed by the intersegmental artery and spinal nerve (**Figure 1**). Spinal ganglia which develop from the neural crest are seen and they are in contact with the neural crest. Ventral and dorsal roots of spinal nerves appear and the spinal nerves develop (**Figure 2**). The notochord is in the centers of sclerotomes and it possesses perinotochordal sheath formed by cells spreading out from the sclerotomes.

In embryos at stage 14 (33 days) caudal sclerotomites are more condensed than their cranial counterparts. The perinotochordal sheath consists of two layers and in this cellular sheath the dense and loose zones appear (**Figure 3**). The loose, cranial zone is the primordium of the vertebral centrum which is the precursor of the vertebral body. Intrasclerotomic fissures are present. Caudal sclerotomites are more condensed and they form intervertebral discs (**Figure 4**) and neural arches as well as their processes. The notochord presents a con-

Table 1. Crown-rump length, development al stage and age in days of investigated embryos

Catalogue number	Crown-rump lenght in mm	Developmental stage	Age in days	Plane of section
B 171	4.0	13	32	Frontal
B 202	4.0	13	32	Horizontal
B 203	5.0	13	32	Sagittal
B 206	5.5	13	32	Horizontal
A 13	7.0	14	33	Sagittal
A 19	7.0	14	32	Horizontal
PJK 20	7.0	15	36	Frontal
PJK 18	9.0	15	36	Frontal
B 69	9.0	15	36	Sagittal
B 181	10.0	16	39	Horizontal
IV	10.0	16	39	Sagittal
B 216	11.0	16	39	Frontal
B 67	12.5	17	41	Sagittal
B 64	13.5	17	41	Frontal
B 68	14.0	17	41	Horizontal
B 128	15.0	18	44	Sagittal
B 208	15.0	18	44	Frontal
Bł 4	15.0	18	44	Horizontal
B 100	16.0	18	44	Sagittal
B 66	16.5	19	46	Horizontal
Z 13	17.0	19	46	Frontal
B 123	17.5	19	46	Sagittal
A 10	18.0	19	46	Horizontal
KA 3	19.0	19	46	Sagittal
Bł 3	20.0	20	49	Sagittal
Z 19	21.0	20	49	Horizontal
A	21.5	20	49	Frontal
B 127	23.5	21	51	Sagittal
A 4	23.5	21	51	Frontal
Z 2	25.0	22	53	Horizontal
B 114	27.0	23	56	Sagittal
Kub 2	28.0	23	56	Frontal
B 177	28.5	23	56	Horizontal
Bł 11	29.0	23	56	Frontal

tinuous, unconstricted rod surrounded by the perinotochordal sheath. It is widest in the lower cervical and upper thoracic parts.

It has to be pointed out that the term centrum is used for the central part of the vertebra and it ossifies from the primary center. The body of adult vertebra includes the centrum and a small part of the neural arch which unites at the neurocentral joint. The neural arch then is more extensive than adult vertebral arch. During stages 13 and 14 the vertical diameter of both sclerotomites is equal.

In embryos at stage 15 (36 days) and 16 (39 days) intrasclerotomic fissures disappear. Dense and loose zones of sclerotomes are well demarcated. This marked distinction between cranial and caudal sclerotomites is seen particularly in the cervical and thoracic parts (**Figure 5**). The notochord is not of uniform diameter throughout and is thicker in future discs. The perichordal centra surrounded by loosly distributed cells form the primordium of the vertebral body (**Figure 6**). In these centra cell density increases laterally in the vicinity of the spinal nerve. This is particularly evident in embryos at stage 17 (41 days), (**Figure 7**). In embryos at stage 15 in the annulus fibrosus of the intervertebral disc two zones may be distinguished. The medial zone, larger and close to the nucleus pulposus is formed of

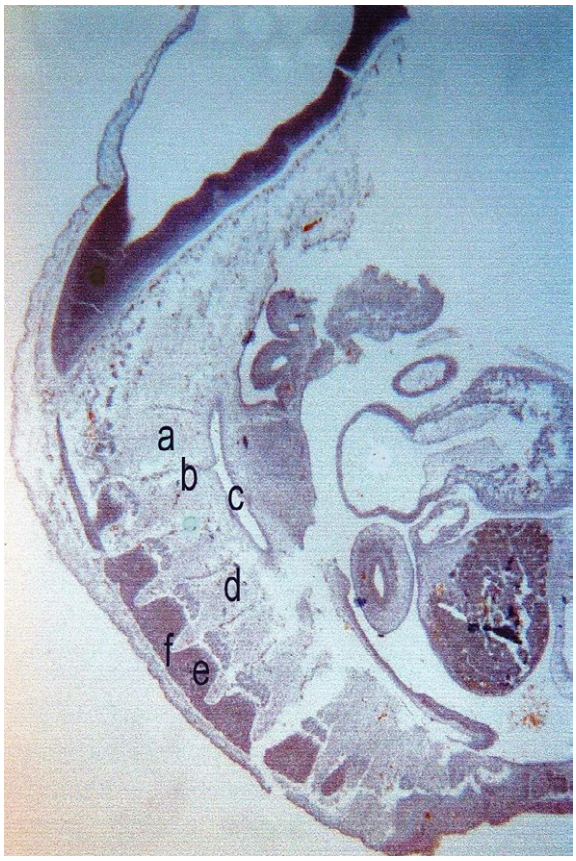

Figure 1. Sagittal section of human embryo at stage 13. Bodian`s protargol, x 100. a – dense zone of sclerotome, b – loose zone of sclerotome, c – dorsal aorta, d – intersegmental artery, e – spinal ganglion, f – neural crest

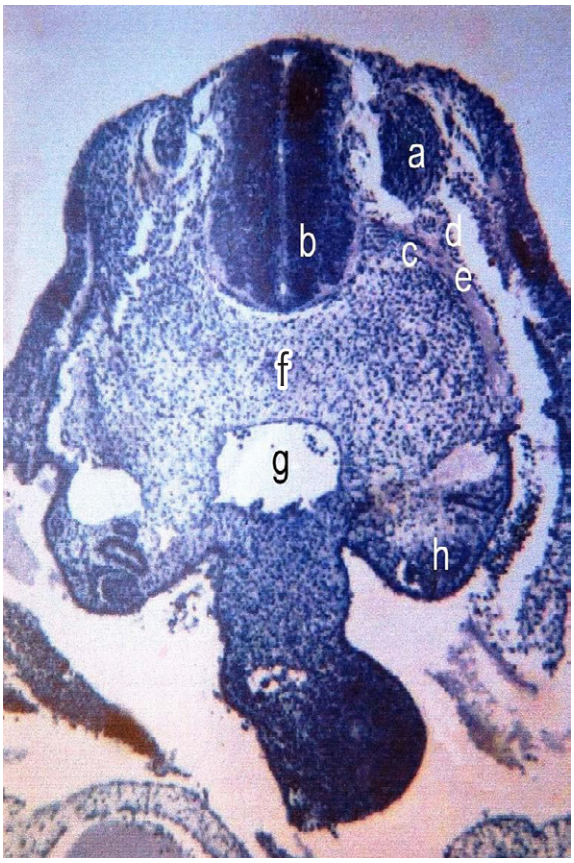

Figure 2. Horizontal section of embryo at stage 13. H+E, x 100. a – spinal ganglion, b – spinal cord, c – ventral root, d – dorsal root, e – spinal nerve, f – notochord with perinotochordal sheath, g – dorsal aorta, h – mesonephros

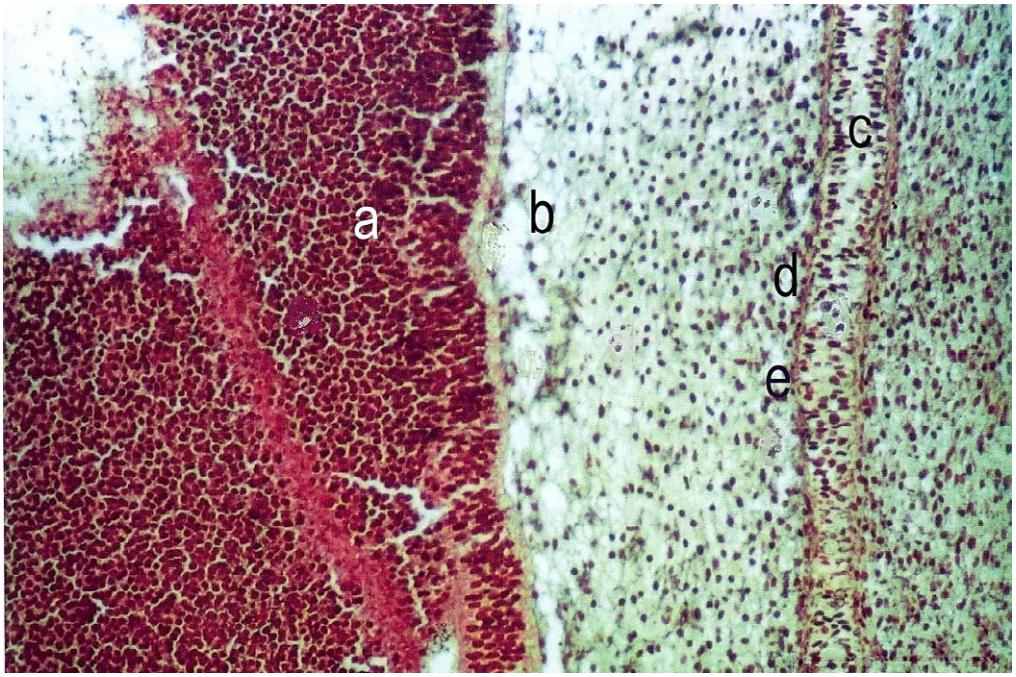

Figure 3. Sagittal section of embryo at stage 14. Bodian`s protargol, x 300. a – spinal cord, b – primary meninx, c – notochord, d – dense zone of perinotochordal sheath, e – loose zone of perinotochordal sheath

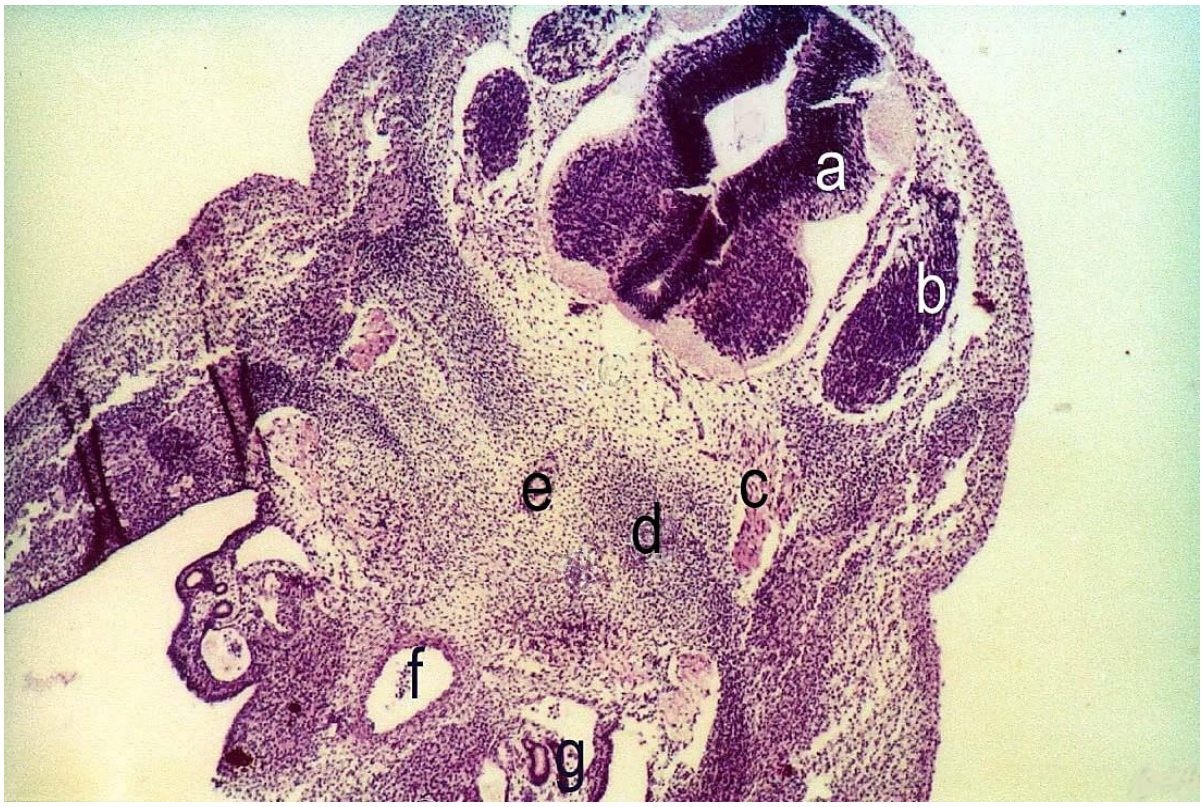

Figure 4. Horizontal section of embryo at stage 14. Cresyl violet, x 100. a – spinal cord, b – spinal gangion, c – spinal nerve, d – intervertebral disc, e – notochord, f – dorsal aorta, g – mesonephros

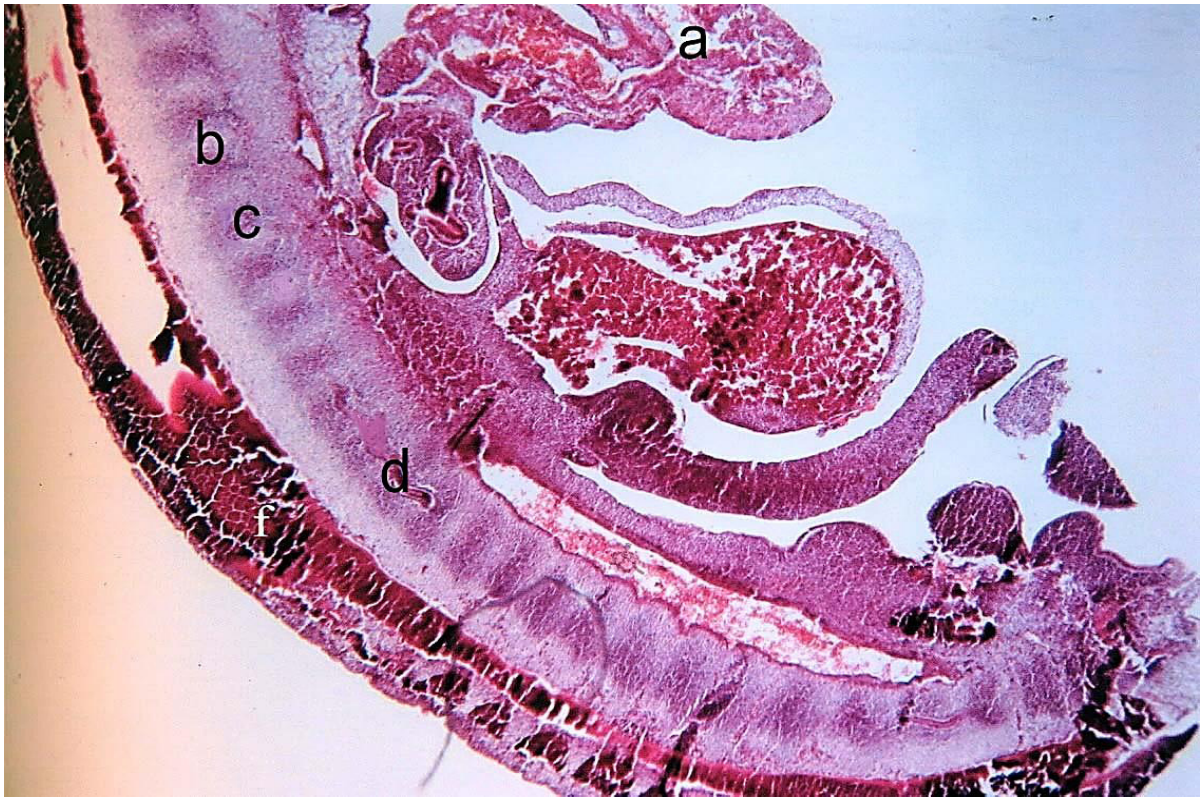

Figure 5. Sagittal section of embryo at stage 15. H+E, x 40. a – heart, b – centrum of vertebra, c – intervertebral disc, e – notochord, f – spinal cord

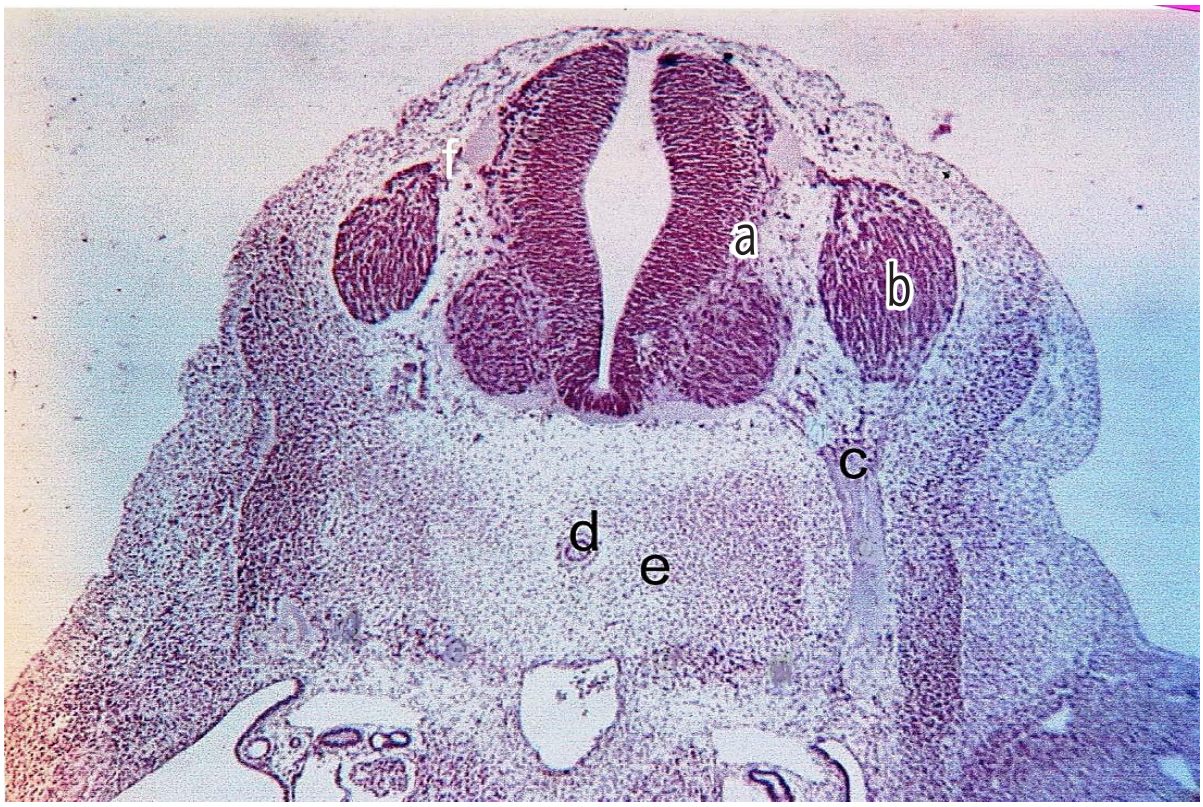

Figure 6. Horizontal section of embryo at stage 15. Bodian`s protargol, x 100. a – spinal cord, b – spinal ganglion, c – spinal nerve, d – notochord, e – centrum of vertebra

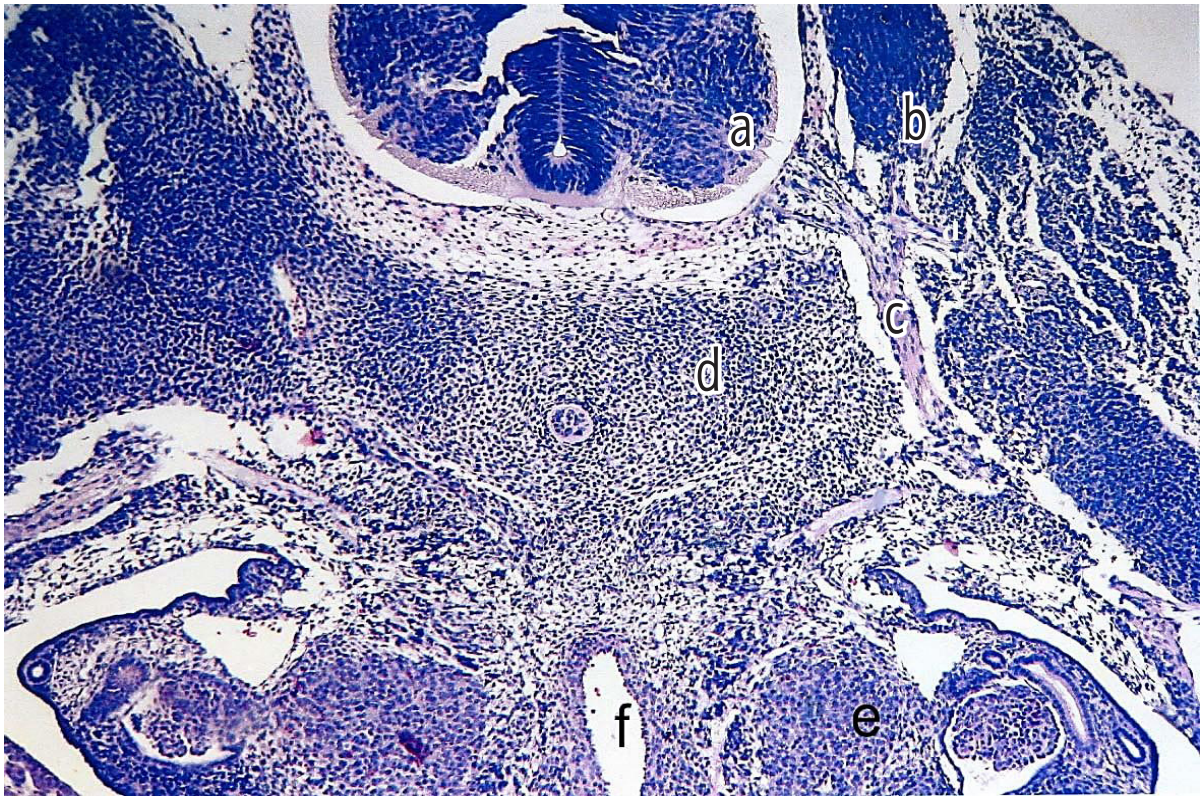

Figure 7. Horizontal section of embryo at stage 17. H+E, x 100. a – spinal nerve, b – spinal ganglion, c – spinal nerve, d – centrum of vertebra, e – suprarenal gland, f – aorta

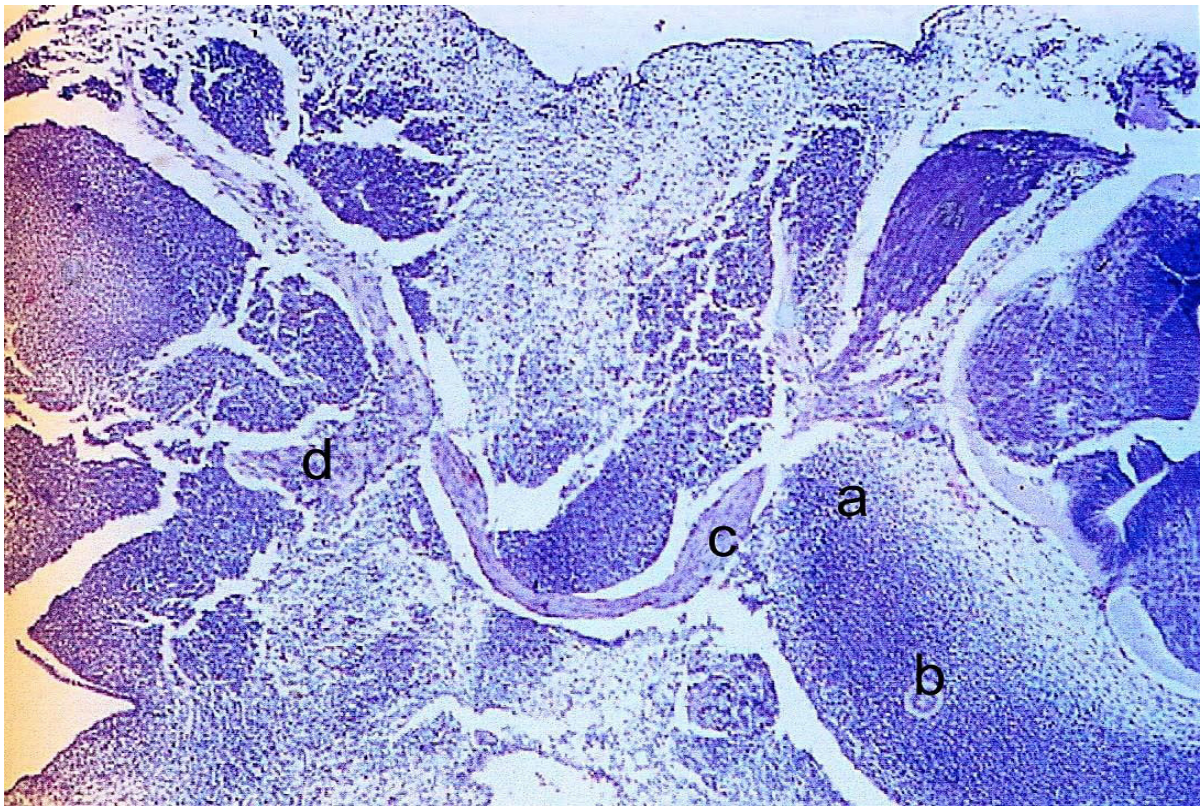

Figure 8. Horizontal section of embryo at stage 15. Cresyl violet, x 100. a – annulus fibrosus, b – nucleus pulposus around the notochord, c – spinal nerve, d – upper limb

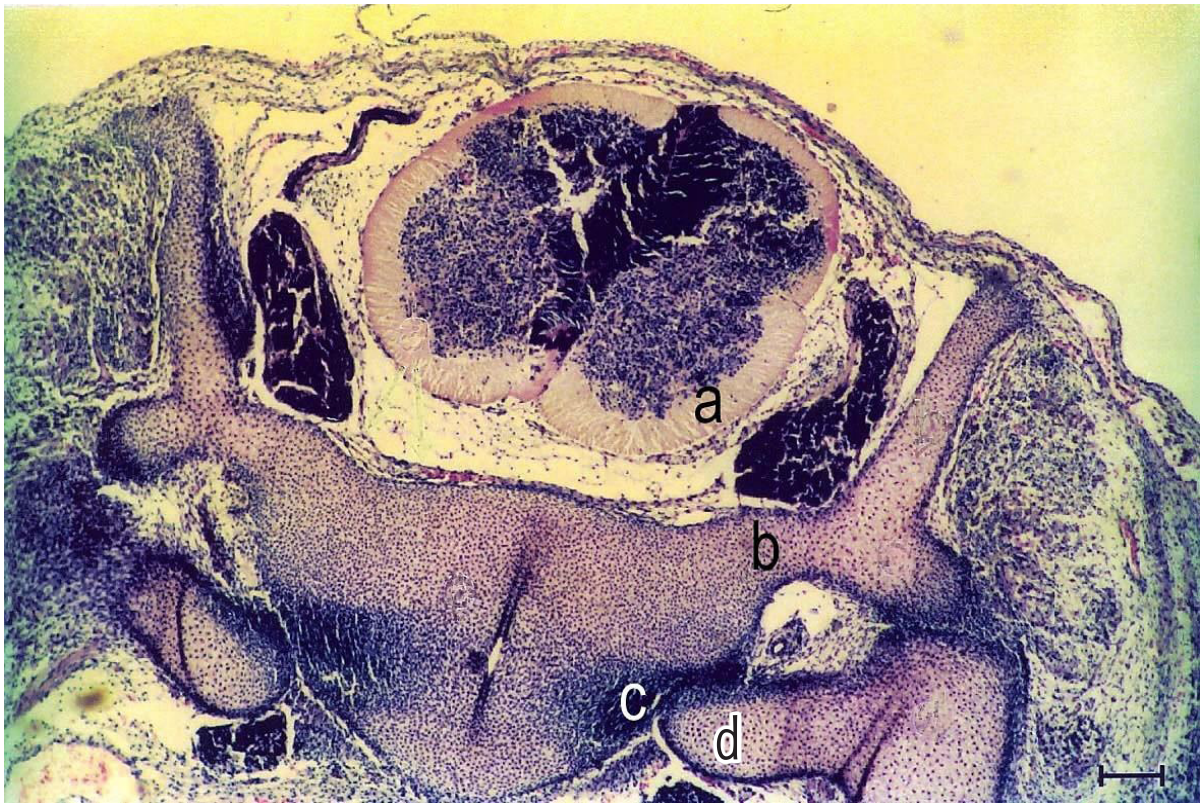

Figure 9. Horizontal section of embryo at stage 19. Cresyl violet, x 100. a – spinal cord, b – neural arch, c – annulus fibrosus, d – head of rib

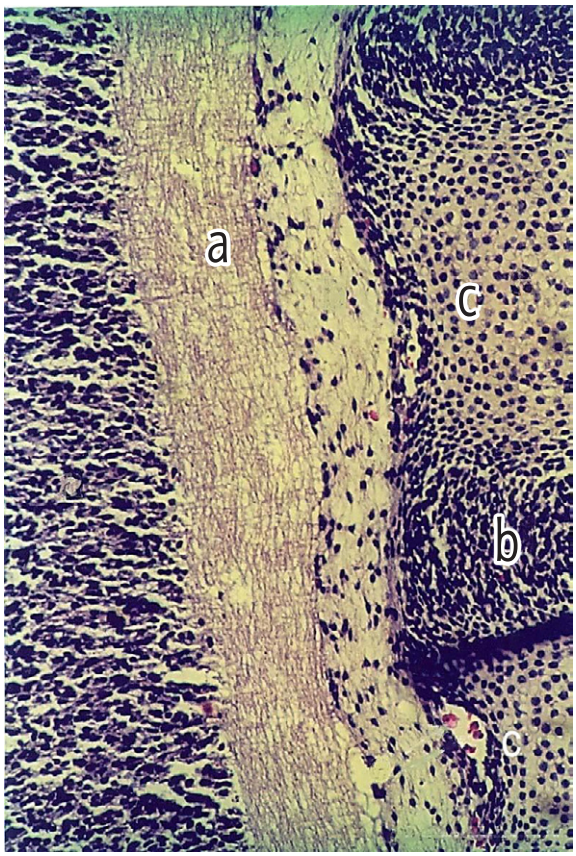

Figure 10. Sagittal section of embryo at stage 19. Cresyl violet, x 400. a – spinal cord, b – annulus fibrosus, c – vertebral body

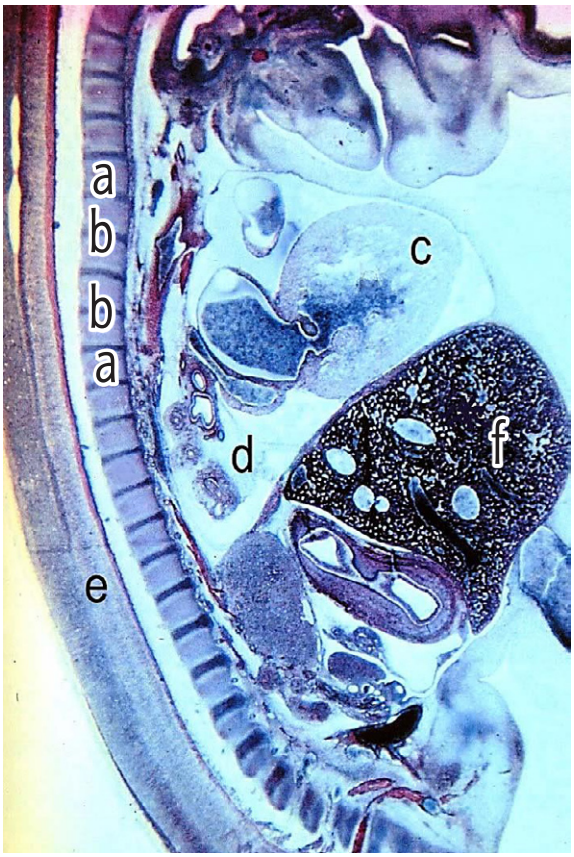

Figure 11. Sagittal section of embryo at stage 19. Mallory stain, x 25. a – vertebral body, b – intervertebral disc, c – heart,d – lung, e – spinal cord, f – liver

densely packed cells. In the lateral zone, which is smaller, cells are more loosely packed and arranged in circular rows. These rows are particularly evident in the lateral margins of the annulus fibrosus (**Figure 8**). Such arrangement is considered as the initial stage of lamellar structure of the annulus fibrosus. It may be concluded then that the laminar structure of the intervertebral disc begins in embryos at stage 15 in the cervical and thoracic parts. The height of the intervertebral disc differs in its posterior and anterior parts. The disc is of the same vertical diameter as the vertebral body in the posterior part and decreases toward the anterior part where it measures 40% of the whole vertebral unit.

With advancement of embryonic development this lamellar arrangement of the annulus fibrosus proceeds from periphery of the disc toward nucleus pulposus and in embryos of stage 19 (46 days) it is distinct in the whole annulus fibrosus (**Figures 9, 10**). In sagittal section of the vertebral column in embryos of stage 19 the intervertebral discs are narrow as compared to vertebral bodies and they are bulging anteriorly and posteriorly (**Figure 11**). The vertical diameter of the intervertebral disc in the cervical, thoracic and lumbar parts of the vertebral column constitutes one third of the height of the vertebral body. At the level of the intervertebral disc the notochord presents cellular aggregations. By the end of the embryonic period the nucleus pulposus shifts dorsally.

Discussion

The development of the vertebral column is preceded by that of the somites and the notochord [17]. The development of the individual vertebrae begins with a Shh-mediated induction by the notochord cells on the somite in order to form the sclerotome. Other signaling pathways which participate in formation of vertebrae include the BMP, WNT, Pax1 and HOX signaling systems [3, 18, 19]. Pax1 gene plays an important role and it is continuously expressed during the development of the intervertebral disc in embryogenesis. This gene encodes transcriptional factors that regulate and take part in vertebrae formation. It is believed that Pax 1 and the subsequent formation of the intervertebral

disc is an important factor in maintaining the segmental character of the vertebral column [20–22].

Several investigators have described the contribution of the loose and dense zones of sclerotomes to formation of the vertebrae and intervertebral discs. Bardeen [23] concluded that the vertebral body and intervertebral disc develop from both sclerotomites. In Prader's theory of vertebral resegmentation, condensed caudal and loose cranial sclerotomites are formed and both differentiate into vertebral body and disc [11, 12]. This theory was also supported by Sensenig [13] and Peacock [15].

More recently the theory of resegmentation of sclerotomes has been denied [14, 17, 24] and it is postulated that the dense zones give rise to the intervertebral discs and take part in the formation of ribs and neural arches, whereas the loose zones and perinotochordal sheath form vertebral body.

The key structure in the development of the intervertebral disc is the notochord, which provides the template for the development of the disc [15, 25, 26]. This rod-like axial structure induces differentiation of mesoderm into sclerotomes.

In the present study it was shown that in the early stages (13 and 14) the notochord is a continuous structure of the same diameter in the loose and dense zones of sclerotomes. Beginning from stage 15 (36 days) the notochord is progressively thicker in the dense sclerotomite which gives origin to intervertebral disc. The period of formation of laminae in the annulus fibrosus during development markedly differs among investigators.

Peacock [15] who expressed age of embryos and fetuses basing on length observed intervertebral expansions of the notochord in embryos at 21 mm what corresponds to Carnegie stage 21 (51 days). The concentric arrangement of cells in the annulus fibrosus he observed in embryos at 10 mm what corresponds to Carnegie stage 16 (39 days).

It has to be stressed that the lamellar arrangement of the annulus fibrosus is accompanied by expansions of the notochord in the intervertebral disc. Such expansions we observed in the present study in embryos of stage 15 (36 days).

O'Rahilly and Meyer [14] noted that the intervertebral discs were evident peripherally as the annuli fibrosi during embryonic period but they do not give precise stage during which differentiate laminae in the annulus fibrosus.

Lohse et al [27] wrote that the annulus fibrosus and nucleus pulposus of the intervertebral disc were identified by 6 months of age and were similar in structure to those of the adult.

The present study performed on staged human embryos with precisely determined age proved that the laminar structure of the annulus fibrosus begins early in the sixth week of embryonic development.

With completion of growth, the vertical diameter of the intervertebral discs diminishes. It was shown by Popova-Latkina [28]. She found that to the end of sixth week the intervertebral disc is one third the height of the adjacent vertebral body. At the beginning of the third month the disc is only one sixth the height of the vertebral body.

In the present investigations in embryos of seventh week the intervertebral disc was one third the height of the vertebral body.

Brandner [29] investigated values of the vertebral body and intervertebral disc index in newborns and adolescents. He found that an index between the disc and the next lower vertebral body height decreases significantly after the first month and is almost stable until 12 years when it decreases once more.

Acknowledgements

Conflict of interest statement
The authors declare that there is no conflict of interest in the authorship or publication of contribution.

Funding sources
There are no sources of funding to declare.

References

1. Pattappa G, Li Z, Peroglio M, Wismer N, Alini M, Grad S. Diversity of intervertebral disc cells: phenotype and function. J Anat. 2012;221:480–496.
2. Humzah MD, Soames RW. Human intervertebral disc: structure and function. Anat Rec. 1988;220:337–356.
3. Henrikson HB, Brisby H. Development and regeneration potential of the mammalian intervertebral disc. Cells Tissues Organs. 2013;197(1):1–13.
4. Grunhagen T, Wilde G, Soukane DM. Nutrient supply and intervertebral disc metabolism. J Bone Joint Surg Am. 88 (suppl 2):30–35.
5. Roberts S, Evans H, Trivedi J, Menage J. Histology and pathology of the human intervertebral disc. J Bone Joint Surg Am. 2006;88(suppl 2):10–14.
6. Nosikova YS, Santerre JP, Grynpas M, Gibson G, kandel RA. Characterization of the annulus fibrosus – vertebral body interface: identification of new structural features. J Anat. 2012;221:577–589.
7. Buckwalter JA. Aging and degeneration of the human intervertebral disc. Spine. 1995;20:1307–1314.
8. Mwale F, Roughley P, Antonion J. Distinction between the extracellular matrix of the nucleus pulposus and hyaline cartilage: a requisite for tissue angineering of intervertebral disc. Eur Cell Mater. 2004;8:58–63.

9. Pezowicz CA, Robertson PA, Broom ND. Intralamellar relationships within the collagenous architecture of the annulus fibrosus imaged in its fully hydrated state. J Anat. 2005;207:299–312.
10. Yu J, Winlove PC, Roberts S. Elastic fibre organization in the intervertebral discs of the bowine tail. J Anat. 2002; 201:465–475.
11. Prader A. Die Frühembryonale Entwicklung der menschlichen Zwischenwirbelscheibe. Acta Anat. 1947;3:68–83.
12. Prader A. Die Entwicklung der Zwischenwirbelscheibe beim menschlichen Keimling. Acta Anat. 1947;3: 115–152.
13. Sensenig EC. The early development of the human vertebral column. Contr Embryol Carneg Instn. 1949;33: 21–41.
14. O'Rahilly R, Meyer DB. The timing and sequence in the development of the human vertebral column during the embryonic period proper. Anat Embryol. 1979;157: 167–176.
15. Peacock A. Observations on the pre-natal development of the intervertebral disc in man. J Anat. 1951;85: 260–274.
16. Pezowicz CA, Robertson PA, Broom ND. The structural basis of interlamellar cohesion in the intervertebral disc wall. J Anat. 2006;208:317–330.
17. O'Rahilly R, Benson DR. The development of the vertebral column. In: Bradford DS, Hensinger RM, editors. The pediatric spine. New York, Thieme; 1985; 3–17.
18. Day TF, Guo X, Garret-Beal L, Yang Y. Wnt/beta-catenin signaling in mesenchymal progenitors controls osteoblast and chondrocyte differentiation during vertebrate skeletogenesis. Dev Cell. 2005;8:739–750.
19. Mundy C, Yasuda T, Kinumatsu T, Yamaguchi Y, Iwamoto M, Enomoto-Iwamoto M, et al. Synovial joint formation requires local Ext1 expression and heparan-sulfate production in developing mouse embryo limbs and spine. Dev Bbiol. 2011;351:70–81.
20. DiPaola CP, Farmer JC, Manova K, Niswander LA. Molecular signaling in intervertebral disc development. J Orthop res. 2005;23:1112–1119.
21. Risbud MV, Schaer TP, Shapiro IM. Toward an understanding of the role of notochordal cells in the adult intervertebral disc: from discord to accord. Dev Biol. 2010;239: 2141–2148.
22. Mansouri A, Hallonet M, Gruss P. Pax genes and their roles in cell differentiation and development. Cur Opin Cell Biol. 1996;8(6):851–857.
23. Bardeen CR. The development of the thoracic vertebrae in man. Amer J Anat. 1905;4:163–174.
24. Grzymisławska M, Woźniak W, Łupicka J, Skórzewska A. The development of the vertebral column in human embryos during fifth week (developmental stages 13–15). Now Lek. 2012;81(6):596–604.
25. Fleming A, Keynes RJ, Tannahill D. The role of the notochord in vertebral column formation. J Anat. 2001;199: 177–180.
26. Hunter CJ, Matyas JR, Duncan NA. Cytomorphology of notochordal and chondrycytic cells from the nucleus pulposus: a species comparison. J Anat. 2004;205:357–362.
27. Lohse CL, Hyde DM, Benson DR. Comparative development of thoracic intervertebral discs and intra-articular ligaments in the human, monkey, mouse and cat. Acta Anat. 1985;122:220–228.
28. Popova-Latkina NV. Etwicklung der Zwischenwirbelscheiben und der Chorda in der Embryonalzeit beim Menschen. Anat Anz. 1967;121(5):518–536.
29. Brandner ME. Normal values of the vertebral body and intervertebral disc index during growth. Radiology. 1970; 110(3):618–627.

Acceptance for editing: 2015-02-10
Acceptance for publication: 2015-03-30

Correspondence address:
Małgorzata Grzymisławska
Department of Anatomy
Poznan University of Medical Sciences
6 Święcicki Str., 60-781 Poznań, Poland
phone: +48 61 8546564
fax: +48 61 8546568
email: malgorzatagrzymislawska@ump.edu.pl

ORIGINAL PAPER

DOI: https://doi.org/10.20883/medical.e14

Assessment of nutritional status and feeding methods in patients with inflammatory bowel disease

Ewelina Swora-Cwynar[1], Jacek Karczewski[2], Anna Musiał, Marian Grzymisławski[1], Emilia Marcinkowska[1], Agnieszka Dobrowolska[3], Dorota Mańkowska-Wierzbicka[3]

[1] Chair and Department of Internal, Metabolic Diseases and Dietetics, Poznan University of Medical Sciences, Poland
[2] Chair of Biology and Environmental Protection, Laboratory of Transplantation Immunology, Poznan University of Medical Sciences, Poland
[3] Chair and Department of Gastroenterology, Human Nourishment and Internal Diseases, Poznan University of Medical Sciences, Poland

ABSTRACT

Aim. The aim of this study was to evaluate diet of patients with IBD, their nutritional status and potential differences as compared to diet of healthy persons.

Material and methods. The examination included the patients of Internal, Metabolic Diseases and Dietetics Ward and Gastroenterology, Internal Diseases and Human Nutrition Ward of Heliodor Święcicki Clinic in the Poznan University of Medical Sciences in Poznan. The criterion allowing participation into the study involved a diagnosed ulcerative or Crohn's disease, basing on histopathology and radiological examination. The study was conducted on 50 patients, 25 women and 25 men. The control of group included 50 persons, 30 women and 20 men, potentially healthy and occupationally active. In the study the authors' own questionnaire was used, which contained questions related to diet and evaluating conditions of nutrition, based on the results of anthropometric measurements and selected biochemical parameters.

Results. The study documented that IBD affected diet, which proved to be distinct from that of potentially healthy person. Course of the disease reduced some laboratory parameters in serum: decreased levels of total protein were detected in 40% patients, reduced levels of albumin in 28% patients and of haemoglobin in 72% patients with IBD. Most of patients introduced some modifications and dietary restrictions to reduce the symptoms and prolong remission. Presentation of such attitudes, indicates a need for an effective multidirectional education, which should affect a conscious decision making about the diet. The Body Mass Index and evaluation of nutrition demonstrated that IBD predisposed development of malnutrition: as compared to the control group, 28% of the patients manifested underweight.

Keywords: inflammatory bowel disease, nutritional status, feeding methods

Introduction

Development of IBD most frequently is noted in young individuals, in their second or third decade of life, or in the period of gaining education and occupational activity, during fulfilment of personal carrier. Manifestation of unpleasant intestinal signs and general symptoms as well as chronic character of the diseases negatively affects daily functioning of the patients. Complications from other organs and the relapsing, frequently severe and unpredictable course of the disease significantly affects quality of patients' life. An important element of the therapy involves dietetic care, which promotes remission process, alleviates signs/symptoms of the disease, prevents against their development and balances off the already existing alimentary deficits. An appropriately balanced alimentation, adapted to the rate of metabolic transformations and supplementing

deficits of nutrients improves efficacy of pharmacotherapy, reduces frequency of complications, duration of hospital stay and of rehabilitation.

Alimentary treatment as an integral part of therapy in IBD

Introduction of alimentary treatment in the course of IBD is important due to co-existing alimentary disturbances (**Table 1**). Most frequently, a disturbed nutriture is observed, particularly in patients with Crohn's disease. The principal causes of malnutrition include:

- Reduced food consumption (fear of relapse in the disease, apprehension that sings/symptoms of the disease will develop, like abdominal pain, diarrhoiea, nausea);
- Disturbed absorption (resections, reduced absorbing surface), augmented intestinal loss, administered drugs, reduced resting energy expenditure [1].

The dietetic management depends on stage of the disease: a distinct alimentation is provided to patients during remission and during exacerbation of the disease [4].

Aim

The study aimed at evaluation of nutrition and manner of alimentation in non-specific bowel diseases and in particular:

- Effect of the disease on alimentary habits of patients with Crohn's disease or ulcerative colitis.
- Evaluation of nutriture as related to selected anthropometric parameters and indices.
- Identification of differences in nutriture between the examined group of patients and the control group.
- Alimentary education, the exponent of awareness in patients with IBD.

Material and methods

The studies aimed at evaluation of nutriture and manner of alimentation in patients with IBD were conducted in the period from October, 2009 to April, 2014.

The examined group included patients staying in the Ward of Internal Diseases, Metabolic Diseases and Dietetics and the Ward of Gastroenterology, Human Alimentation and Internal Diseases, Poznan University of Medical Sciences in Poznan. The criterion qualifying patients to participation in the studies involved diagnosis of ulcerative colitis or Crohn's disease. The inquiring person clarified doubts upon filling by a patient of the questionnaire, containing data on the purpose of the study and an assurance that the study manifests an anonymous character. Anthropometric measurements were conducted in the wards during registration of the patients. Results of laboratory tests were obtained from information charts describing stay in the hospital.

Control group included potentially healthy and occupationally active individuals. The participants unaided filled the questionnaire and conducted anthropometric measurements.

The study was performed on 50 patients, including 25 with ulcerative colitis and 25 with Crohn's disease. The patients included 23 women and 27 men. Mean age of the studied population amounted to 36 ± 14.9 years At the time of examination the eldest participant was 70 years old, the youngest one was 18 years old.

The control group included 50 persons, 30 women and 20 men. Mean age of the group amounted to

Table 1. Frequency of feeding disturbances in patients with nonspecific intestinitis [2, 3]

Deficiencies of nutrients in nonspecific intestinitis		
Feeding disturbance	Prevalence of IBD (%)	Prevalence of Crohn's disease (%)
Loss in body weight	18–62	65–75
Lactose intolerance	25–65	30–40
Hypoalbuminaemia	25–50	25–80
Anaemia – Deficiency of folic acid – Deficiency of vitamin B12 – Deficiency of iron	22–68 5–20 8–30 30–80	60–80 50–79 16–48 10–44
Deficit-induced bone diseases	0–15	24–39
Deficit of essential fatty acids	0–2	2–5
Deficit of calcium	0–46	20–60
Deficit of magnesium	2–55	30–68
Deficit of zinc	12–52	42–92

38 ± 14.3 years. At the time of examination the eldest participant was 63 years old, the youngest one was 22 years old.

The study took advantage of our own questionnaire, consisting of two parts: registration data and evaluation of alimentation manner. The latter was based on replies to 24 closed type questions, in one case with the potential to supplement the reply. Questions related to age, sex and basic anthropometric parameters manifested an open character. Nutriture was evaluated in the basis of anthropometric measurements, such as current body weight and results of biochemical tests conducted during hospital stay of a patient.

Analysis of the collected material and processing of results took advantage of Microsoft Excel 2007 and Statistica 10 Statsoft. The results were regarded statistically significant at $p \leqslant 0.05$. The results were presented below in the form of tables and graphs.

Results

Analysis of alimentation manner

Frequency and regularity of meal consumption during day

A significant difference was disclosed in the number of meals consumed by IBD patients and by control individuals ($p = 0.00001$). Patients with IBD consumed more meals in the course of a day: as many as 50% of the patients declared consumption of 5 or more meals in the clourse of a day. Such a number of meals was declared by only 8% of healthy individuals. Over 50% less patients than healthy individuals declared consumption of only three meals per day (20% vs 42%). No patient consumed only two meals per day (**Figures 1, 2**).

In the case of regularity of meal consumption a significant difference was noted in times of their consumption: constant vs variable times ($p = 0.0004$). 84% ($n = 42$) of questioned IBD patients consumed meals in constant time points while only 50% ($n = 25$) of healthy individuals declared regularity of meal consumption (**Figure 3**).

Consumption of fruits and vegetables

Most of participants, either patients or healthy ones (84% and 70%, respectively), consumed fruits and vegetables once or twice a day. The lowest number of either examined population or control individuals (2% and 6%, respectively) consumed fruit and vegetables 5 or more times a day (**Table 2**).

Among questions related to manifestation of IBD and effects of the form in which fruits and vegetables were consumed (raw/processed) a statistically significant difference was demonstrated ($p = 0.00077$). The principal difference was related to consumption of vegetables and fruits in their raw form: as compared to healthy individuals, IBD patients less frequently consumed raw vegetables (70% vs 26%). A similar difference, although less pronounced, wase detected in consumption of raw fruits: only 50% patients as compared to 84% healthy individuals preferred fruits in their raw form. On the other hand, close to 100% respondents, both healthy ones and IBD patients (94% vs 98%), consumed vegetables in their processed form (**Figure 4**).

Types of preferred corn products

Analysis of preferred corn products demonstrated a significant difference in type of selected bread between the examined group and the control group ($p = 0$). 80% patients declared preference for purified corn products, which were selected by only 20% members of the control group. The sick individuals also less frequently than control individuals preferred mixed corn products (14% vs 32%). The lowest proportion of patients (6%) preferred full grain corn products, which were most frequently selected by healthy individuals (48%) (**Figure 5**).

Consumption of dairy produces

Most of inquired participants consumed dairy products. However, three-fold more numerous members of the examined group declared abstention from consumption of dairy products than members of the control group (18% vs 6%). Only 14% patients pointed to

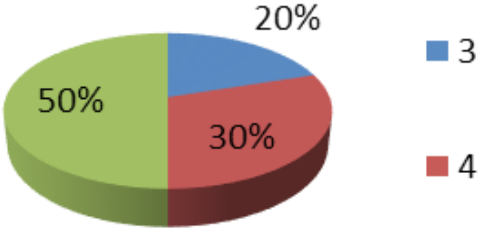

Figure 1. Frequency of meal consumption in examined group

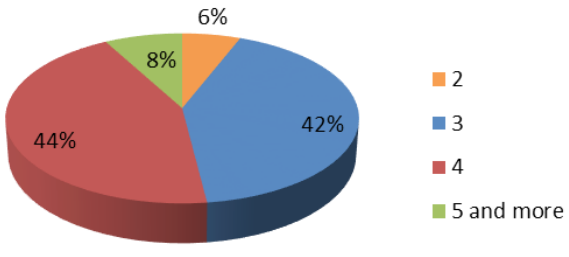

Figure 2. Frequency of meal consumption in control group

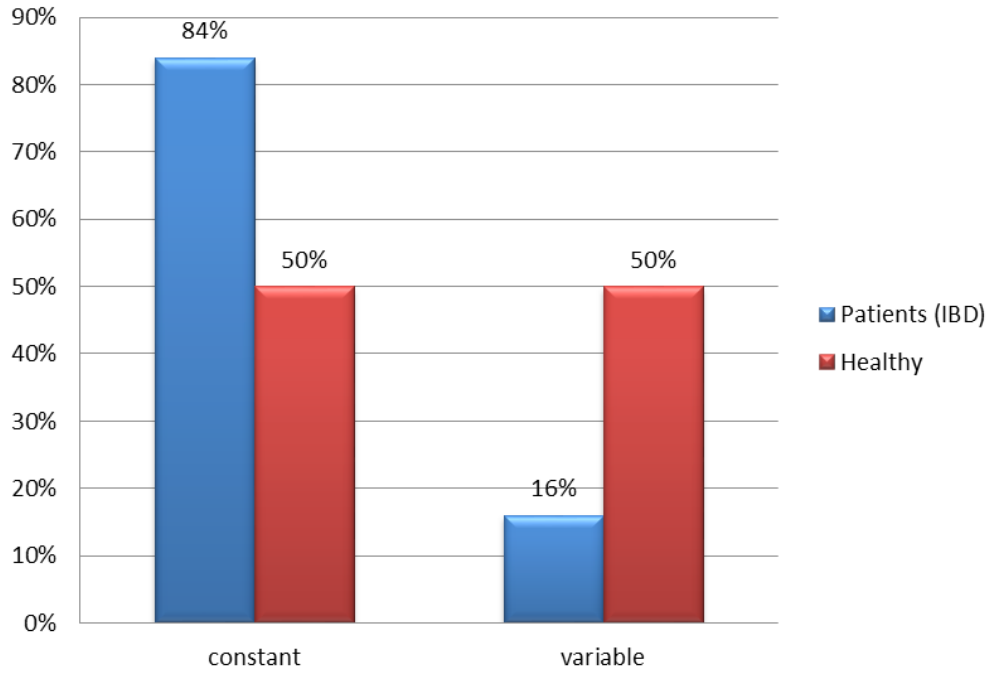

Figure 3. Manifestation of the disease (IBD) and regularity of meal consumption

Table 2. Frequency of consuming vegetables and fruits versus incidence of the disease

	Frequency of consuming vegetables and fuits		
	1–2 x/day	3–4 x/day	⩾ 5x/day
Examined group	84% (n = 42)	14% (n = 7)	2% (n = 1)
Control group	70% (n = 35)	24% (n = 12)	6% (n = 3)

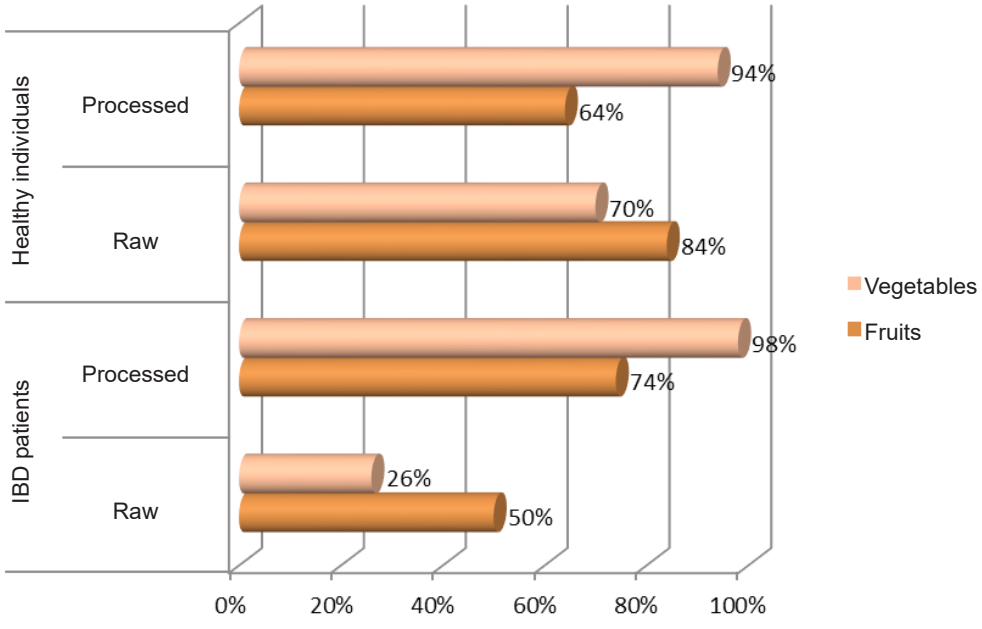

Figure 4. Form of consumed fruits and vegetables

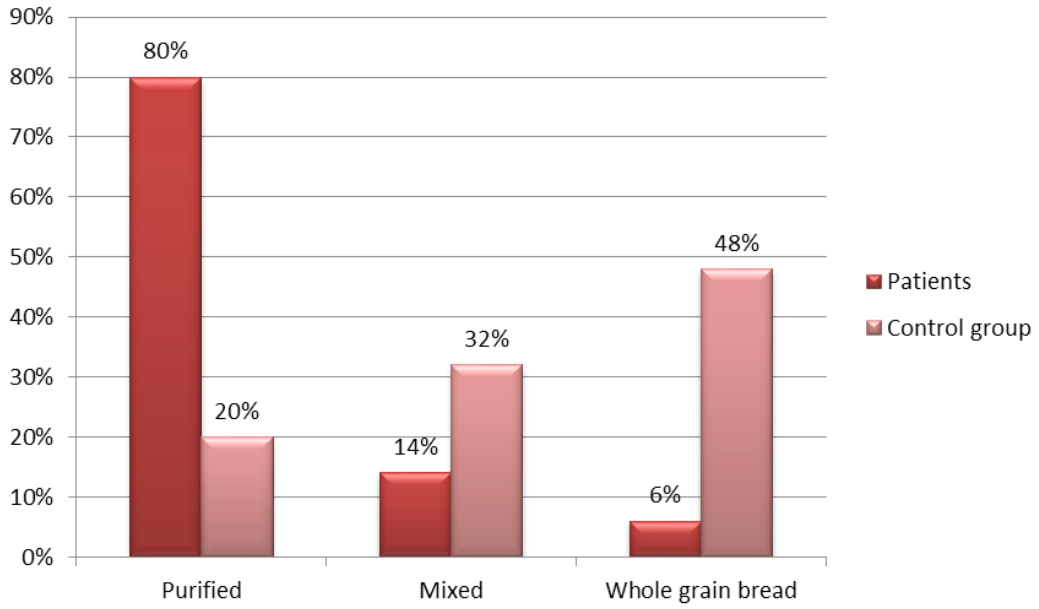

Figure 5. Preferred type of bread

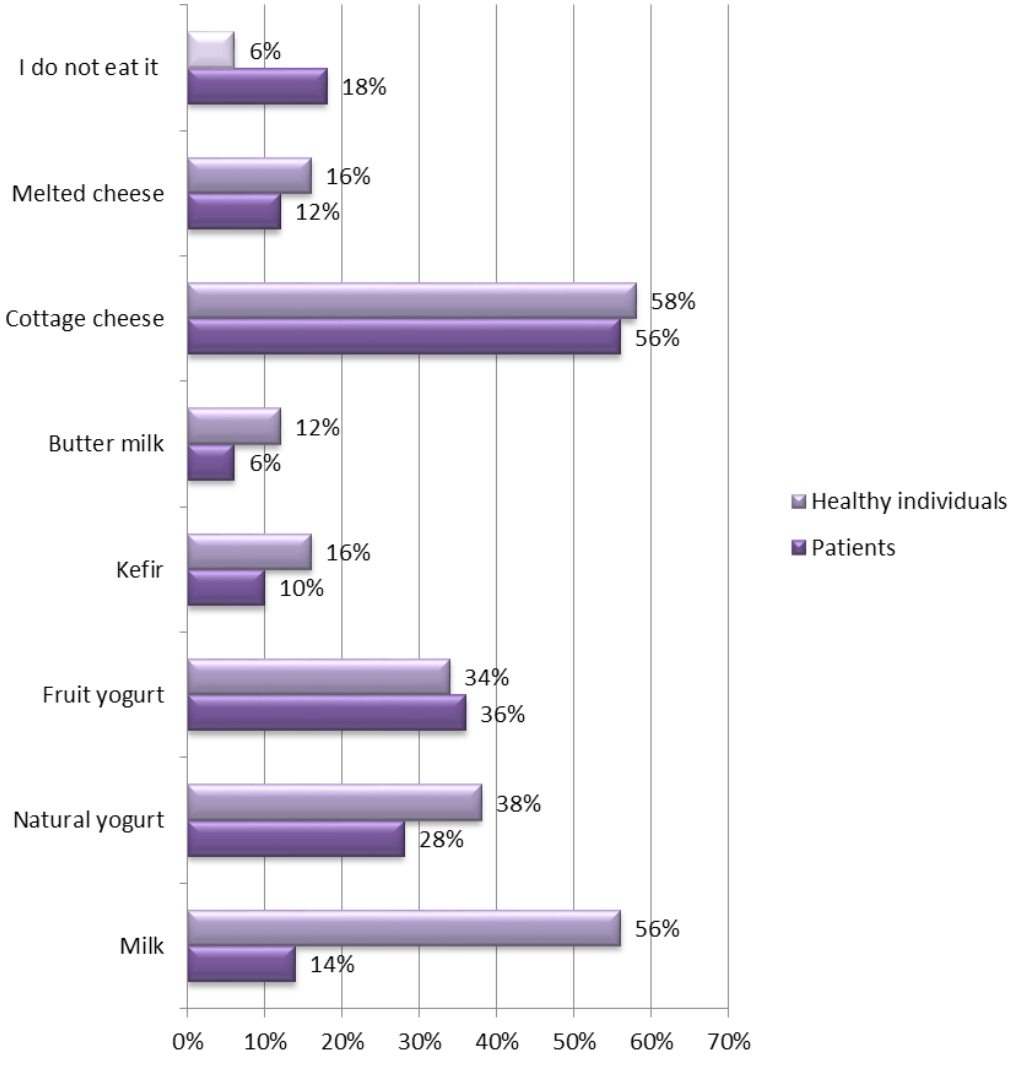

Figure 6. Types of consumed dairy produce among healthy individuals and those suffering of IBD

Table 3. Frequency of consuming dairy produces in control group and examined group

	Manifestation of IBD	
Frequency of consuming dairy produces	Yes	No
< 1 once per day	15 (37%)	14 (30%)
once per day	18 (44%)	17 (36%)
2–3 times per day	7 (17%)	15 (32%)
4 and more times a day	1 (2%)	1 (2%)
Total	41 (100%)	47 (100%)

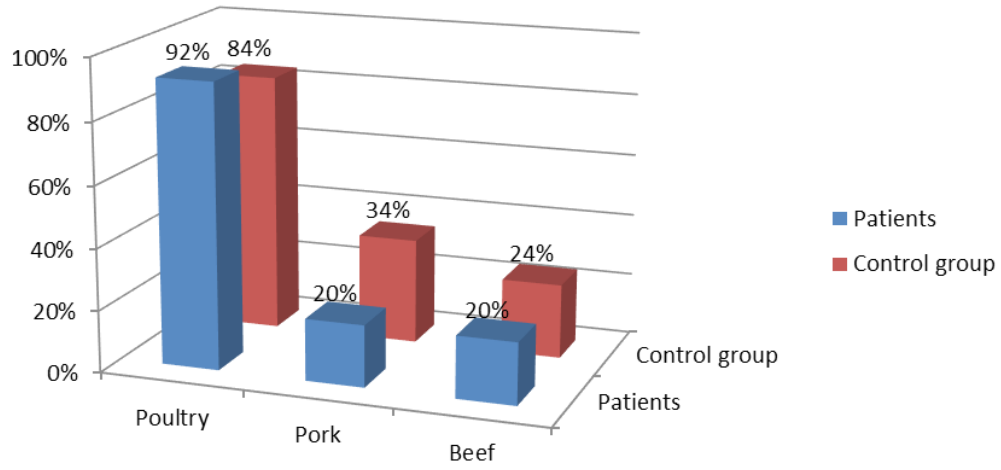

Figure 7. Preferred types of meat consumed in the examined group and among control individuals

Table 4. Manifestation of IBD and type of preferred culinary processing

	Manifestation of IBD		
Type of culinary processing	Yes	No	
Boiling	35 (70%)	16 (32%)	p=0.033
Steaming	21 (42%)	19 (38%)	
Roasting	15 (30%)	16 (32%)	
Frying	9 (18%)	29 (46%)	

introduction of milk to their diet in contrast to healthy individuals of whom as many as 56% respondents declared consumption of milk. Among dairy products a similar proportion of patients and members of the control group most frequently selected cottage cheese (56% vs 58%). Among fermented milk products a similar number of sick and healthy respondents selected fruit yogurts (36% vs 34%). On the other hand patients suffering of IBD less frequently than healthy individuals declared consumption of natural yogurt (28% vs 38%), kefir (10% vs 16%), and butter milk (6% vs 12%) (**Figure 6**).

Analysis of frequency manifested by milk product consumption showed that 44% patients declaring consumption of milk products consumed them once daily, 37% did so less frequently than once a day. Only 7% of patients consumed milk products 2–3 times a day and only 2% of them 4 or more times a day. In the group of healthy individuals, similarly like among patients, most numerous respondents declared cinsumption of milk products once a day (36%) but, in contrast to patients, as many as 32% consumed milk products 2–3 times a day (**Table 3**).

Consumption of meat

Among meat types, most patients (92%) most frequently selected poultry, consumption of beef and pork was declared by the same fraction of respondents (20% vs 20%). Comparing preferences as to the type of consumed meat in the examined group and the control group, sick individuals were found to select pork less frequently than healthy individuals did (20% vs 34%). In cases of poultry and beef a similar proportion of the two groups of respondents declared their consumption (**Figure 7**).

Statistical analysis of preferred culinary processing demonstrated effect of the processing on manifestation of IBD (p = 0.033). In the examined group of patients

most frequently boiling (70%), steaming (42%), roasting (30%), and least frequently frying (9%) was used. On the other hand in the case of control group the distribution of culinary processing was inverse: boiling and steaming were used least frequently (32% each), followed by roasting (38%) and frying (46%) (**Figure 8, Table 4**).

Use of lipids

A statistically significant proved to be the difference between control group and the examined group of patients in the type of lipids used for smearing of bread ($p = 0.023$). In cases of fats used to smear bread more respondents in the examined group of patients than in the control group smeared bread with butter (78% vs 48%). On the other hand, soft margarine was more frequently used by healthy individuals than by the patients (32% vs 18%). In either examined group of patients or healthy individuals none of the participants declared that they did not smear bread (**Figure 9**).

Consumption of condiments: coffee and alcohol

A significant difference was disclosed between the examined group and the control group in consumption

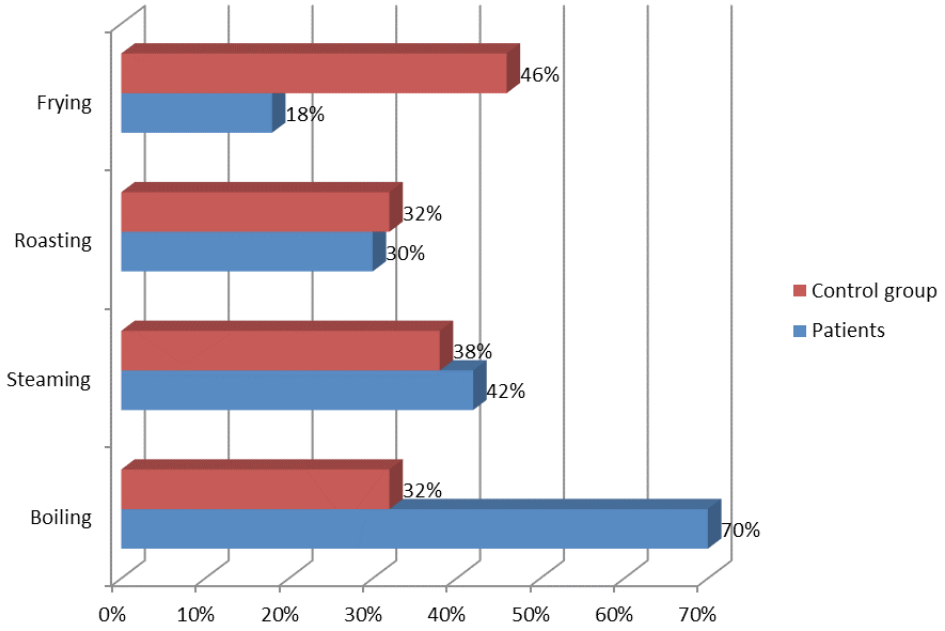

Figure 8. Manifestation of IBD versus preferred culinary processing

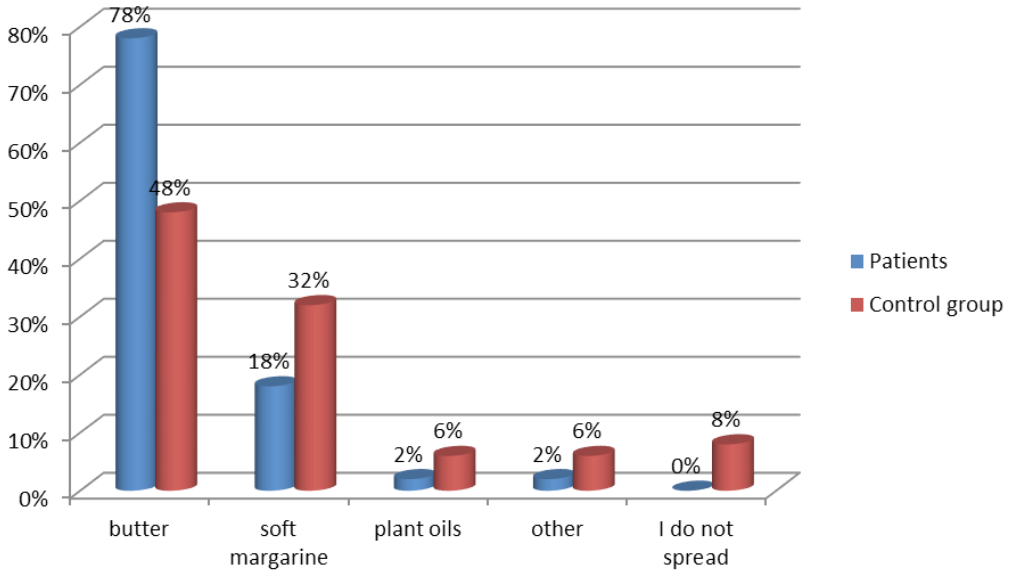

Figure 9. Type of fat used to spread over bread

of coffee by respondents (p = 0.0001). Among participants of the examined group of patients 60% declared that they drink no regular or instant coffee as compared to healthy individuals of whom 78% declared drinking coffee (**Figure 10**).

Statistical analysis demonstrated that IBD affected frequency of alcohol consumption by participants (p = 0). The affected individuals less frequently consumed alcohol than healthy individuals: 62% of patients declared that they do not drink alcohol while only 8% of healthy individuals provided such a reply. In the examined group of patients none of the participants declared alcohol consumption 4–6 x/week or daily. On the other hand in the control group daily alcohol consumption was declared by 2% participants while 8% consumed alcohol 4–6 x/week (**Figure 11**).

Use of sharp spices

Presence of the disease significantly affected use of sharp spices by participants (p = 0). Among the participants, patients with IBD less frequently used sharp spices than healthy individuals (14% vs 62%) (**Figure 12**).

Evaluation of nutriture

Nutriture was evaluated on the basis of BMI index and results of selected laboratory tests performed on the patients. On the other hand, results of laboratory tests in the control group were accepted to represent normal values (**Table 5**).

In the studied population mean value of body mass index (BMI) amounted at the level of 21.4 ± 4.03 kg/m^2. The lowest value of the index involved 14.94 kg/m^2, and the highest one was 30.4 kg/m^2. In the control group the mean was higher, 23.8 ± 3.5 kg/m^2, with the minimum value of 18.7 kg/m^2 and the maximum value of BMI amounting to 32.5 kg/m^2 (**Table 5**).

Statistical analysis demonstrated effect of IBD on frequency of malnutrition among participants (p = 0.0049). Deficit of body weight was disclosed in 28% patients manifesting BMI at the level of <18.5kg/m^2**.** In the control group, on the other hand, none of the participants manifested malnutrition. The normal nutriture was more frequently manifested by healthy participants than by the patients (70% vs 48%) although differences in frequency of manifestation of an excessive body weight in the examined group of patients and the control individuals were less pronounced (24% vs 30%) (**Table 6**).

A statistically significant negative correlation was demonstrated between age and frequency of malnutrition (r=-0.014). Deficits in body weight were manifested less frequently in individuals older than 40 years of age (10%), as compared to patients between 18 and 30 years of age and between 31 and 40 years of age (respectively: in 44% and 29%) (**Table 7**).

In the examined group of patients the highest proportion of patients with deficit of body weight involved patients at the age of 18 to 30 years (43%), followed by those between 31 and 40 years of age (29%), and, the least frequent, patients older than 40 years of age

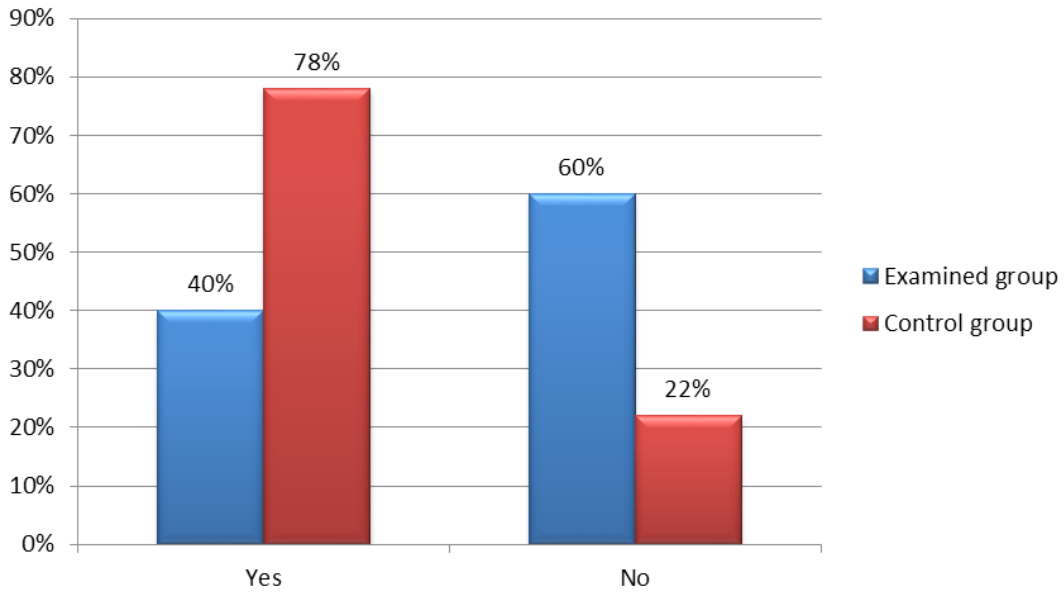

Figure 10. Consumption of regular coffee and instant coffee in control and examined group

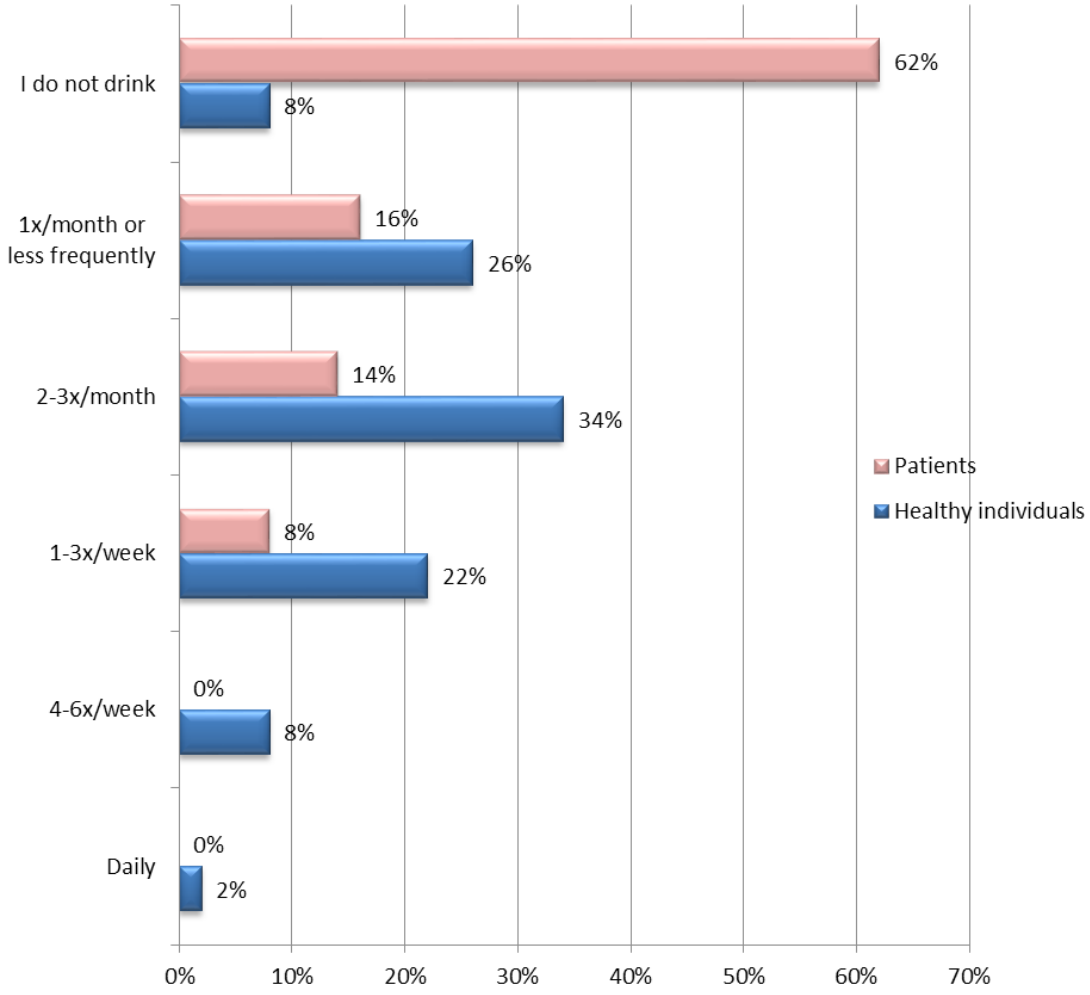

Figure 11. Frequency of alcohol consumption among healthy individuals and the patients

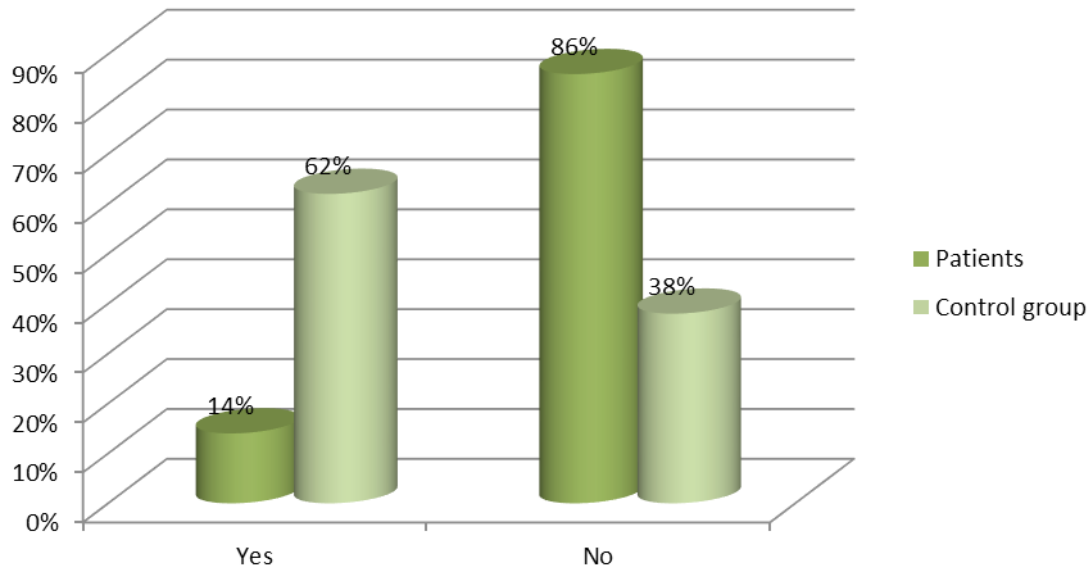

Figure 12. Use of sharp spices versus manifestation of IBD

Table 5. Value of BMI – index in examined group and control group

	Number (n)	Mean	Minimum	Maximum	Standard deviation
Examined group	50	21.4 kg/m^2	14.9 kg/m^2	30.4 kg/m^2	4.03
Control group	50	23.8 kg/m^2	18.7 kg/m^2	32.5 kg/m^2	3.5

Table 6. Frequency of malnutrition

BMI	Examined group		Control group		
	Number (n)	Percent (%)	Number (n)	Percent (%)	
< 18.5 kg/m^2 – malnutrition	14	28	0	0	p = 0.0049
18.5–24.9 kg/m^2 – normal level	24	48	35	70	
⩾ 25 kg/m^2 – excessive body weight	12	24	15	30	

Table 7. Nourishment of patients and their age

BMI	Age (years)						
	18–30		31–40		> 40		
	Number (n)	Percent (%)	Number (n)	Percent (%)	Number (n)	Percent (%)	
< 18.5 kg/m^2 – malnutrition	10	44	2	29	2	10	r = -0.014
18.5–24.9 kg/m^2 – normal level	11	48	5	71	8	40	
⩾ 25 kg/m^2 – excessive body weight	2	8	–	–	10	50	
Total	23	100	7	100	20	100	

Table 8. Normal levels of selected laboratory indices

Index	Normal level
Albumin	3.5–5.2 g/dl
Total protein	6.4–8.3 g/dl
Haemoglobin	13.5–17.2 g/dl

(10%). Among healthy individuals no body mass deficits were detected in any age group. Most numerous patients at the age of 31 to 40 years manifested normal body weight (71%), and an excessive body weight in group older than 40 years of age (50%). In turn, in the control group participants at the age of 18 to 30 years most frequently manifested a normal or excessive body weight (88% and 56%, respectively) (**Figures 13, 14**).

In the examined group of patients 28% manifested a lowered level of albumins (< 3.5 g/dl). Their average level amounted to 4.74 ± 0.7g/dl, with the lowest value of 2.42 g/dl, and the highest one of 6.73 g/dl (**Tables 9, 10**).

In the case of total protein level, the lowest value amounted to 4.16 g/dl, and the highest one to 8.47g/dl. The mean level was 6.83 ± 1.5g/dl. However, in 40 % participants its level was lower than the normal value (6.4 g/dl) (**Tables 11, 12**).

Concentration of haemoglobin in serum in 72% examined individuals was exceedingly low (< 13.5 g/dl). The minimum level noted oscillated around 11.9 ± 1.13 g/dl, the maximum level was 15.9 g/dl, the average level was 6.7 g/dl (**Tables 13, 14**).

A positive correlation was disclosed between albumin level and value of BMI (r = 0.28). In 71% of examined individuals manifesting normal body weight value of albumin level remained in the normal range. In patients with deficit of body weight the level of albumin equally frequently remained below or within the normal range (50% vs 50%). Among individuals with an excessive body weight, 73% of them manifested normal level of albumin (**Table 15**, **Figure 15**).

Discussion

The manner of nutrition in patients with confirmed diagnosis of IBD is an integral element of therapy, sig-

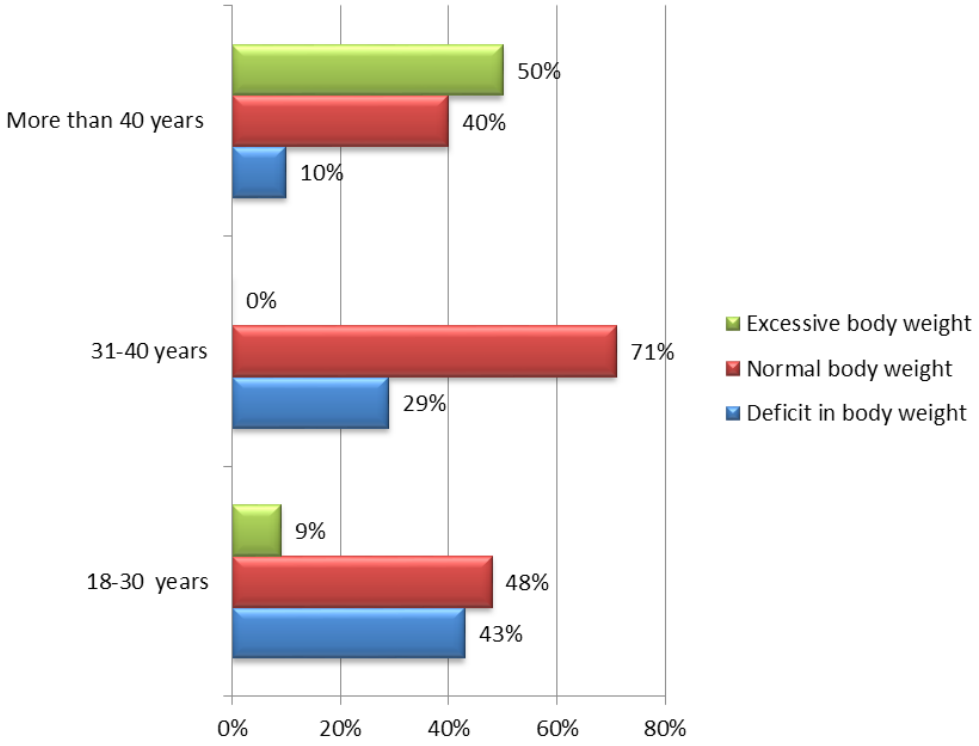

Figure 13. Disturbed alimentation versus age in the examined group

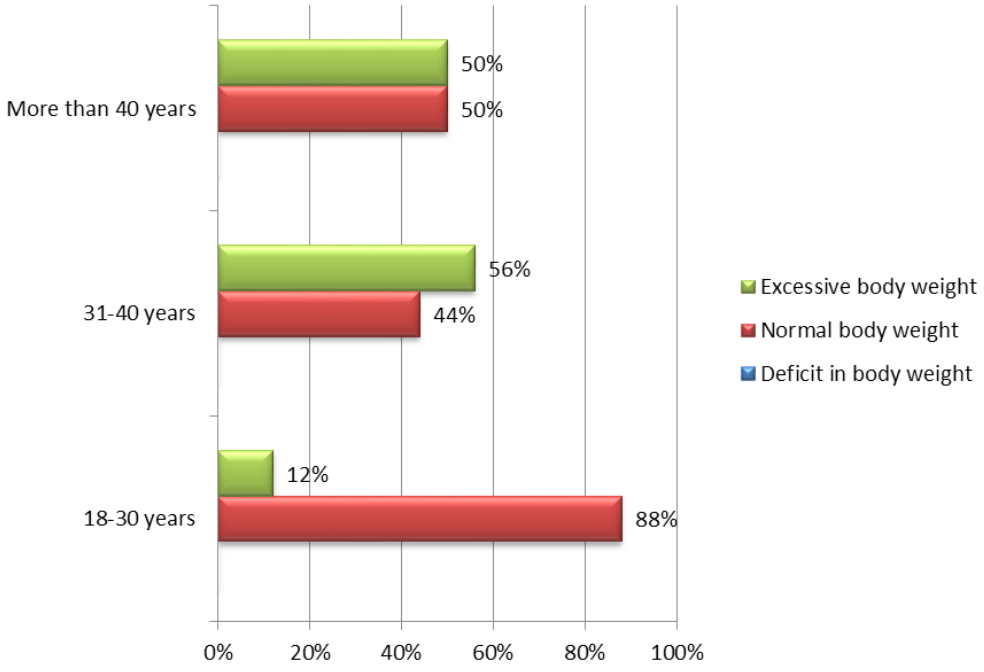

Figure 14. Disturbed nourishment and age structure of control group

nificantly affecting course of the disease and efficacy of its treatment. Errors in alimentation may abbreviate the period of remission and accelerate its relapse. Therefore, the need arises to introduce individual modifications and restrictions in diet.

In the current knowledge no data are available allowing definition of clear alimentation recommendations for patients with inflammatory disease of intestines. Nevertheless, the patients frequently state that specific food products influence clinical signs/symptoms of the disease. Cohen AB et al. described patients' opinions on advantages and harms related to selected food products, related to the disease course. The food types which, according to the questionnaire,

Table 9. Level of albumin

	Number (n)	Mean	Minimum	Maximum	Standard deviation
Albumin	50	4.74 g/dl	2.42 g/dl	6.73 g/dl	0.7

Table 10. Level of albumin as related to the norm

Albumin (g/dl)	Examined group
	Number (n)
Below 3.5	14 (28%)
Norm 3.5–5.2	33 (66%)
Above 5.2	3 (6%)

Table 11. Level of total protein

	Number (n)	Mean	Minimum	Maximum	Standard deviation
Total protein	50	6.83g/dl	4.16g/dl	8.47g/dl	1.5

Table 12. Total protein as related to the norm

Total protein (g/dl)	Examined group
	Number (n)
Below 6.4	20 (40%)
Norm 6.4–8.3	3 (6%)
Above 8.3	27 (54%)

Table 13. Haemoglobin level

	Number (n)	Mean	Minimum	Maximum	Standard deviation
Haemoglobin	50	11.9 g/dl	6.7g/dl	15.9	1.13

Table 14. Level of haemoglobin as compared to the mean

Haemoglobin (g/dl)	Examined group
	Number (n)
Below 13.5	36(72%)
Norm 13.5–17.2	14(28%)
Above 17.2	0

were selected by the patients as improving clinical signs/symptoms included yogurt, rice and bananas. Food products which were indicated as deteriorating the signs/symptoms included leafed vegetables, spicy food, fruits, nuts, fried meals, milk, red meat, popcorn, milk products, alcohol, food rich in cellulose, maize, fatty meals, seeds, grains of coffee [5].

In the study, half of the patients were demonstrated to consume five or more meals per day, which in cases of IBD is recommended The same number of meals was declared by only 8% of examined healthy individuals. Only 20% of the patients declared consumption of three meals per day. However, taking into account the healthy Polish population, 76% individuals consume three meals a day [6]. The number of meals per pay involves an important aspect since the more numerous they are, the more favourably they affect function of alimentary tract and the less they embark the inflamed intestinal mucosa. In this way the contained in them nutrients may be used to a maximum extent for body needs.

The patients are increasingly aware that apart from higher number of meals per day it remains important to plan them and to consume them in stable time points of the day. Eighty-four % of examined patients consumed meals in stable times of a day and only every other healthy individual indicated regularity of meal consumption.

In the examined populations everybody declared consumption of fruits and vegetables. However, current norms of alimentation recommend consumption of fruits and vegetables five times a day. Most of the patients

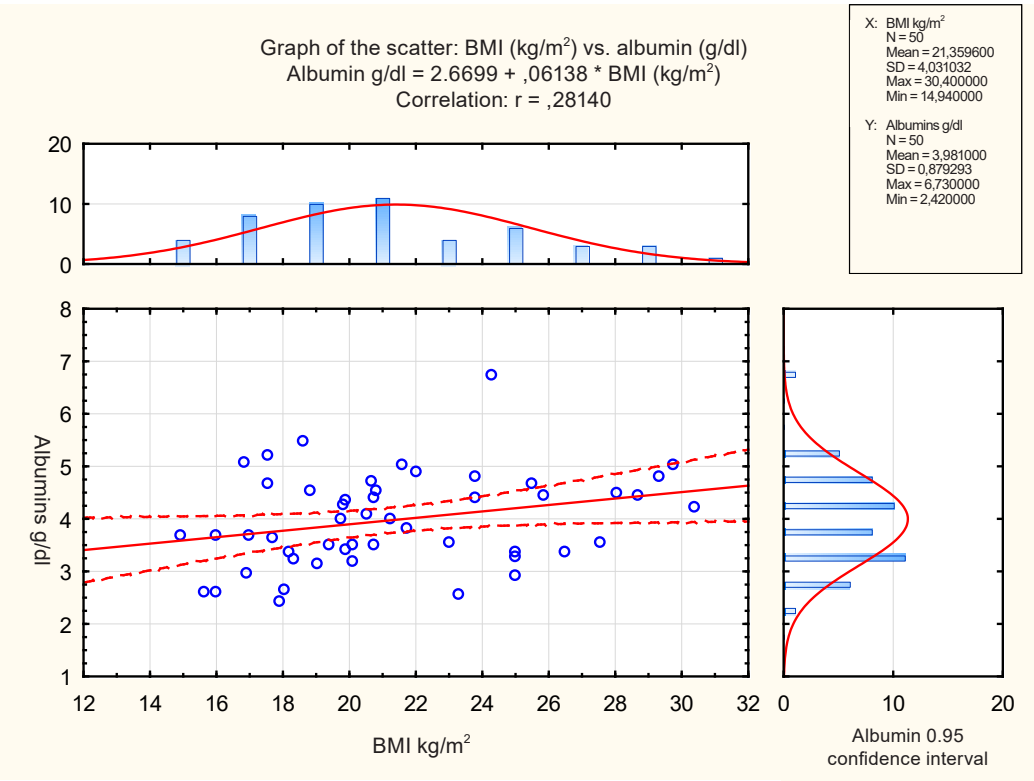

Figure 15. Relationship between BMI and albumin level in patients with NChZJ

Table 15. Relationship between albumin level and BMI index in examined group

BMI	Albumin			
	Deficiency	Norm	Excess	Total
Deficiency	7 (50%)	7 (50%)	–	14 (100%)
Norm	4 (19%)	15 (71%)	2 (10%)	21(100%)
Excess	4 (27%)	11(73%)	–	15(100%)

(84%) and of healthy individuals (70%) declared their consumption only once or twice a day. Only 14% of the patients consumed fruits and vegetables 3 to 4 times a day and only a single patient consumed them 5 or more frequently a day. Taking into account healthy Polish population [6] only 5% of healthy individuals consume fruits more than once a day.

Even if patients with IBD declare consumption of fruits and vegetables, most of them take advantage of fruits and vegetables in their processed form. Processing of vegetables and fruits (boiling, rubbing through a strainer, peeling) causes that they are less irritant to pathologically altered intestinal mucosa and they are digested easier. Such actions allow that valuable vitamins (vitamins K, C, B group vitamins, folic acid, β-carotene) and mineral components (Ca, K, Mg, Zn, Fe, Se) are absorbed by the body, the need for which in the patients is higher than in healthy individuals due to, i.a., losses resulting from diarrhoea. Therefore, most of the patients (98%) prefer vegetables in their processed form and only 26% of them in their raw form, as compared to healthy population in which 70% individuals consume vegetables in their raw form. In cases of fruits, every other persons consumed them in their unprocessed form (50%).

The interviewed patients among the types of bread products most frequently selected purified products (80%). Mixed corn products and full grain products were selected decisively less frequently. This is consistent with recommendations for persons with IBD [7]. The resignation of corn products other than purified ones may reflect intensification of symptoms

(abdominal pain, discomfort, diarrhoea) after their consumption.

Patients are cautious in selection of food products which might intensify their complaints: in the examined group of patients three-fold more numerous members of the examined group declared avoidance of milk products than in the control group (18% vs 6%). Only 14% of patients who declared consumption of milk products introduced milk to their diet, which might reflect fear of unfavourable signs/symptoms. Literature data indicate that frequency of lactose intolerance in ulcerative colitis amounts to 25–65%, and in Crohn's disease 30–40% [3].

The conducted study indicates that 44% of the patients declared consumption of milk products once daily while the same frequency was declared by 36% healthy individuals. In studies by CBOS [6] similar results were obtained: 44% of healthy Polish population consumes milk products every day. The patients less frequently consumed milk products twice to three times a day (7% vs 32%). Upon absence of intolerance, the patients should as frequently as possible introduce milk products to their diet, since this allows them to cover their requirement of calcium, increased as a sequel of steroid therapy. Moreover, milk and milk products supply other valuable components, such as protein of full value and vitamins A, D and group B vitamins.

The obtained data related to type of preferred meat indicate that most of studied patients (92%) selected poultry. Moreover, the patients less frequently than healthy individuals selected pork (20% vs 34%). The patients made an appropriate choice as to an appropriate manner of culinary processing of meat: boiling, steaming and roasting was selected by most respondents (boiling 70%, steaming 42%, roasting 30%). Only 9% of the patients preferred frying, as compared to 42% of healthy examined controls.

The data related to the type of preferred meat and the used processing manner are satisfactory. Most of the patients preferred poultry, the meat rich in protein and containing low level of fat, consistent with principles of easily digested food and appropriate for this group of patients. In addition, the preferred manner of preparing meat for consumption is favourable [8].

Out of the patients who participated in the study more, as compared to healthy individuals, used butter to smear bread (78% vs 48%). This choice resulted perhaps from alimentary habits from the period before development of the disease or from the fact that butter represents the easily digested and best absorbed animal fat.

The conducted study showed that more than every other patient with IBD declared avoidance of regular or instant coffee as compared to healthy individuals, of whom only 22% provided such an reply. Elimination of coffee from diet of affected individuals is justified since it accentuates intestinal peristalsis and may intensify diarrhoea. Such behaviour is consistent with alimentary recommendations for patients with IBD [4]. Nevertheless there exist authors who, on grounds of their own results, found consumption of coffee and infusions of chamomile to represent well tolerated drinks and safe for patients with ulcerative colitis [9].

The examined patients less frequently than healthy individuals consumed alcohol: 38% of examined patients declared consumption of alcohol, as compared to as many as 92% healthy individuals. Study of TNS OBOP demonstrated similar data: 85.5% of Polish adults consume alcohol. Moreover, none of the patients declared consumption of alcohol 4–6 x/week or daily. Reduction of alcohol consumption in IBD is very favourable since alcohol stimulates fermentation processes in intestines and may promote intensification of diarrhoea [10].

The need of an easily digested diet is noticable even in cases of spices: the patients give up application of sharp spices, which unfavourably affect condition of intestinal mucosa, intensifying complaints. Only 14% patients and 62% healthy individuals declared use of sharp spices.

Among the examined population mean value of BMI amounted to 21.4 ± 4.03kg/m^2. On the basis of the index malnutrition was diagnosed in 28% patients. The BMI value within normal range was disclosed in 48% patients, 24% patients manifested an excessive body weight. Literature of the subject presents slightly divergent results related to mean values of BMI. According to results obtained by Zawadzka et al. [11], mean value of BMI in two groups of patients suffering of ulcerative colitis or Crohn's disease oscillates around 20.7 ± 3.08 kg/m^2. In the quoted study, BMI of around 26% patients pointed to malnutrition. According to Poniewierka et al. [12] mean value of BMI in ulcerative colitis amounted to 23.74 ± 4.72 kg/m^2 and in Crohn's disease it was 21.61 ± 3.64 kg/m^2. The BMI value within normal limits was detected in around 62% patients, in 14% patients malnutrition and in 23% an excessive body weight were disclosed. Ripoli J et al. compared nutritive parameters (dietetic, biochemical and anthropometric ones) in patients with ulcerative colitis (n = 65, 24 with active form of the disease, 41 patients in remission) monitored for a period

of one year. The authors detected a marked reduction in BMI, decreased consumption of energy, proteins, lipids, calcium, iron and phosphorus in the group of patients with active disease as compared to patients in remission [13].

Malnutrition in this group of patients tends to manifest complex pathogenesis. The malnutrition results from fear of exacerbation of the disease signs/symptoms causing that the patients in advance eliminate from their diet meals which might intensify gastric and intestinal complaints. Frequently individuals experiencing unfavourable symptoms following consumption of milk in advance eliminate also milk products. Most of them remain unaware that they might introduce to their diet fermented milk products, containing lower amounts of lactose. In addition, the long-term intensified losses of nutrients due to intensified diarrhoea, an intensified catabolism and loss of appetite lead to development of malnutrition.

The studies conducted heretofore demonstrated that the average level of total protein amounted to 6.83 ± 1.5 g/dl. In 40% patients the concentration was below the normal level (6.4 g/dl). The maximum level noted amounted to 8.47 g/dl, the lowest one was 4.16 g/dl. In the study of Zawadzka et al. [11], the mean level of total protein amounted to 6.0 ± 1.04 g/dl in ulcerative colitis and 6.4 ± 0.9 g/dl in Crohn's disease. The amplified losses, a disturbed intestinal absorption and hypercatabolism linked to mediators of inflammatory condition may result in exceedingly low level of total protein.

Mean albumin level in IBD patients amounted to 4.74 ± 0.7 g/dl. More than every other patient (66%) manifested normal albumin concentration, 28% of the patients manifested their decreased level (< 3.5 g/dl). In studies conducted by Zawadzka et al. [11], the mean albumin level amounted to 3.3 ± 0.8 g/dl in ulcerative colitis and 2.9 ± 0.8 g/dl in Crohn's disease. Around 61% of the patients demonstrated an exceedingly low level of albumin. Deficiency of albumin may represent a sequel of the progressing inflammatory condition, leading to reduced synthesis of albumin, their degradation and clear losses in the vascular bed. In the study conducted by Vanis N et al. on 210 patients with IBD in the years of 2010–2012 the authors detected hypoalbuminaemia in 37.6% participants, with a significantly lower albumin level in the group of moderately or high activity of IBD, at $p < 0.05$ (ANOVA) [14].

Concentration of haemoglobin in serum was below the norm (< 13.5 g/dl) in 72% patients even if in none of the participants the exceedingly low concentrations were detected. The mean detected haemoglobin level amounted to 11.9 ± 1.13 g/dl. In the studies of Zawadzka et al. [11] the mean level of haemoglobin in patients with ulcerative colitis amounted to 10.3 ± 3 g/dl/ and in patients with Crohn's disease it was 11.1 ± 1.6 g/dl. According to literature data anaemia affects around 30% of patients with IBD, but some authors quote somewhat higher incidence of anaemia, detecting it even in up to 40% patients with IBD [14, 15]. Aetiopathogenesis of anaemia manifest a complex character and it may reflect chronic loss of blood (overt or occult one) from the damaged intestinal mucosa, erythropoesis disturbed due to abnormal course of immune reactions and undesired effects of drugs [15]. Following a long-term analysis (years of 2009 to 2013) of 410 patients with IBD Koutroubakis IE et al. concluded that anaemia (persistent or relapsing one) correlated with a more aggressive course of the disease and lowered life quality of the patients as compared to patients free of the accompanying anaemia [16].

In order to obtain higher efficacy of treatment and to gain a generally better health in patients with IBD it would be indicated to introduce routine alimentary education worked out by clinical dieteticians.

Conclusions

1. Non-specific inflammatory diseases of intestines determine the manner of patient alimentation and require that dietetic modifications are introduced.
2. Changes in alimentary habits and modification of diet in relation to activity of IBD provide evidence for growing awareness of the patients.
3. In the studied group BMI values and evaluation of nutriture prove that IBD represent a factor promoting development of malnutrition.
4. It was confirmed that course and activity of IBD reduce selected laboratory indices in the serum, such as total protein level, levels of albumin and haemoglobin.

Acknowledgements

KBN nr N N402 482037

Conflict of interest statement
The authors declare that there is no conflict of interest in the authorship or publication of contribution.

Funding sources
There are no sources of funding to declare.

References

1. Poniewierka E. Alimentation in diseases of alimentary tract and in metabolic disturbances [in Polish]. Cornetis, Wrocław; 2010.
2. Adamski Z, Linke K, Samborski W. Biological treatment in dermatology, gastroenterology and rheumatology [in Polish]. Termedia 2nd Edition, 2015; 311–327.
3. Muller-Nothmann SD. Alimentation in non-specific inflammatory diseases of intestines. Falk Foundation e.V., Freiburg; 2007.
4. Ryżko J, Olek A. Dietetic management of non-specific inflammatory diseases of intestines in children [in Polish]. Pediat Współcz. 2001;3:265–269.
5. Cohen AB, Lee D, Long MD, Kappelman MD, Martin CF, Sandler RS, Lewis JD. Dietary patterns and self-reported associations of diet with symptoms of inflammatory bowel disease. Dig Dis Sci. 2013 May;58(5):1322–8. doi: 10.1007/s10620–012–2373–3. Epub 2012 Aug 26.
6. CBOS, Alimentary behaviours and habits in Poles [in Polish]. BS/150/2010.
7. Krzesiek K. Progress in treatment of non-specific intestinitis [in Polish]. Nowa Pediatria. 2002;3:179–184.
8. Ciborowska H, Rudnicka A. Dietetics. Alimentation of healthy and sick humans [in Polish]. PZWL, Warszawa 2009.
9. Langhorst J, Varnhagen I, Schneider SB, Albrecht U, Rueffer A, Stange R, Michalsen A, Dobos GJ. Randomised clinical trial: a herbal preparation of myrrh, chamomile and coffee charcoal compared with mesalazine in maintaining remission in ulcerative colitis – a double-blind, double-dummy study. Aliment Pharmacol Ther. 2013 Sep;38(5):490–500. doi: 10.1111/apt.12397. Epub 2013 Jul 4.
10. TNS OBOP. Consumption of alcohol in Poland in 2012 [in Polish].
11. Zawadzka P, Grzymisławski M. Assessment of malnutrition in a group of patients with inflammatory bowel disease. Gastroenterologia Polska. 2006;13(6):449–453.
12. Poniewierka E, Poniewierka A, Jasiński R. Selected anthropometric indicators in patients with inflammatory bowel disease. Gastroenterologia Polska. 2008;15(6):385–389.
13. Ripoli J, Miszputen SJ, Ambrogini Jr O, Carvalho Ld. Nutritional follow-up of patients with ulcerative colitis during periods of intestinal inflammatory activity and remission. Arq Gastroenterol. 2010 Jan-Mar;47(1):49–55.
14. Vanis N, Mehmedovič A, Mesihovič R, Saray A. Anaemia and inflammatory bowel disease. Prilozi. 2013;34(2):35–42.
15. Radwan P, Radwan-Kwiatek K, Skrzydło-Radomańska B. Rydzewska G.: Anaemia in non-specific intestinitis- aetiopathogenesis, diagnosis and treatment [in Polish]. Prz Gastroenterol. 2010;5:315–320.
16. Koutroubakis IE, Ramos-Rivers C, Regueiro M, Koutroumpakis E, Click B, Schoen RE, Hashash JG, Schwartz M, Swoger J, Baidoo L, Barrie A, Dunn MA, Binion DG. Persistent or recurrent anemia is associated with severe and disabling Inflammatory Bowel Disease. Clin Gastroenterol Hepatol. 2015 Apr 8. pii: S1542–3565(15)00374–2. doi: 10.1016/j.cgh.2015.03.029. [Epub ahead of print].

Acceptance for editing: 2015-02-10
Acceptance for publication: 2015-03-30

Correspondence address:
Ewelina Swora-Cwynar
Katedra i Klinika Chorób Wewnętrznych,
Metabolicznych i Dietetyki
Poznan University of Medical Sciences
49 Przybyszewskiego Str., 60-355 Poznań, Poland
phone: +48 61 8691314
email: eswora@ump.edu.pl

ORIGINAL PAPER

DOI: https://doi.org/10.20883/medical.e15

Alcohol use among medicine and law students in Poland

Wiktor Suchy[1], Agnieszka Gaczkowska[1], Adam Pawelczyk[1], Piotr Pukacki[1], Robert Chudzik[2]

[1] Poznan University of Medical Sciences, Poland
[2] Medical University of Lublin, Poland

ABSTRACT

Introduction. Alcohol, together with drug use such as marijuana, is a major health concern that may influence the life of both doctors and medicine students. It is therefore important to investigate their habits associated with those hazardous behaviors.

Material and methods. A voluntary survey containing 12 questions regarding their drinking habits and marijuana use was sent to law and medicine students from two cities in Poland, Poznan and Lublin. 814 responses were collected and the results were compiled using STATISTICA 10 program.

Results. Mean age of alcohol initiation was revealed to be very similar in all groups at below 16 years of age. Although majority of students drink less than once a week (41% male and 65.7% female), men were found to use alcohol much more frequently and in higher quantities than female students. Half of future doctors would stop at the lowest stage on a proposed alcohol intoxication scale, while 11.6% would venture to the highest, third one. Those values for law students were 36.2% and 26%, respectively. 70% of men and 52.9% of women have tried marijuana. Majority of them smoke less than once a month, but almost a quarter of law students and 15% of medicine students do it at least once a month.

Conclusions. More emphasis should be put on educating future doctors and general public about dangers associated with hazardous drinking and cannabis use. Prevention of such behaviors should be conducted at an age as young as possible.

Keywords: drugs, addiction, hazardous drinking, marijuana.

Introduction

Alcohol is one of the main causes of death in highly developed countries. In Europe, a quarter of deaths among people aged 15–29 years old is linked to alcohol and dangerous behaviors associated with its consumption [1]. Alcohol overuse is commonly associated with pathological population margin. It should be noted that alcohol is dangerous not only among chronic alcohol abusers, but also among casual consumers (binge drinking). Alcohol overuse also favors experimenting with other psychoactive and illegal substances. Medicine students are no exception and their alcohol drinking habits are similar to other people their age. Future doctors with unhealthy habits may be less effective in educating their patients on leading a healthy lifestyle [2]. Alcohol abuse among students may lead to injuries, conflicts, violent behavior, sexual abuse, learning problems and death [3]. Young, active people with above average intelligence tend to have high levels of discipline, which may give them false sense of control over their addictions. This may further weaken their awareness and lead to reckless and dangerous behaviors. Students often don't realize that the level of alcohol intoxication they consider typical for a night out with friends is indicative of alcohol poisoning. This study attempts to evaluate drinking habits of medical students. As future doctors, they should exhibit a high level of knowledge about the influence of alcohol and its chronic and casual abuse, on human

body. At the same time, doctors are often associated with alcohol abuse.

Material and methods

A voluntary survey was conducted in March 2015. Anonymous questionnaires were sent out and placed on internet forums for medical and law students in Poznan and Lublin to fulfill. The survey consisted of 12 questions regarding basic information of the participants (sex, age, university etc.) and their experiences associated with alcohol and marijuana use (**Table 1**). 814 students filled out the questionnaire.

The characteristics of the group are shown in **Table 2**.

The results were compared among medicine and law students. The groups were also divided by the city of residence and of course, male and female participants. Alcohol intoxication levels were suggested based on an artificial scale [5] translated and presented in **Table 3**.

Only stages 2 to 4 were presented as options in the questionnaire.

The results were compiled using STATISTICA 10 program. Group characteristics were created using basic statistics.

Results

Mean age of alcohol initiation was revealed to be 15.62 years for both men and women. Those from the village started later, at age 15.99, as compared to city residents at 15.38 years. This age for medicine doctors turned out to be 15.55 years compared to 15.38 for law students. No significant differences were revealed between any of those groups. An average student has his/her first contact with alcohol being under 16 years old. That's when they graduate from gymnasium and proceed to high school. The transition, associated with new environment, more freedom and influence from older students may facilitate alcohol initiation at this particular age.

Table 4 represents the frequency of alcohol consumption of students in aforementioned groups.

Table 1. Questions in the form

Age	
Sex	Male/Female
Faculty	Medicine/Law
Faculty year	1–6
Residence	– city above 500 000 inhabitants – city between 100 000 and 500 000 inhabitants – city below 100 000 inhabitants – village
University residence	Poznań/Lublin
Age of alcohol initiation	
How often do you drink alcohol	Every day 5–6 times a week 3–4 times a week 1–2 times a week less than once a week
How much alcohol do you consider safe/normal to drink during one evening? * one shot of vodka (50 ml) equals one beer; one shot of vodka equals one drink unless you know your favourite drink contains more shots of vodka	1–2 shots 3–4 shots 5–6 shots 7–8 shots more than 10 shots
What kind of behaviour do you consider indicative of having drunk too much alcohol * In short, when do you think you've had enough to drink	Self control impairment, sluggishness, verbosity Coordination and balance impairment, aggression Sleepiness, deep self control and balance impairment, mumbling, unsteady walk
Have you ever tried marijuana?	Yes/No
If yes, how often do you smoke?	Every day Few times a week Once a week Few times a month Less than once a month

Table 2. Clinical characteristics of the study group

N	814
Sex (M/F)	307/507
Age (years)	22.5 (18–40)
Faculty (medicine/law)	398/416
Year	1st – 152 2nd – 181 3rd – 161 4th – 126 5th – 103 6th – 91
Residence	City above 500 000 inhabitants – 304 City between 100 000 and 500 000 inhabitants – 196 City below 100 000 inhabitants – 206 Village – 108
University residence (Poznań/Lublin)	592/222
Alcohol initiation age	15.46 (6 – 23)
Frequency of alcohol consumption	Less than once a week – 459 1–2 times a week – 247 3–4 times a week – 74 5–6 times a week – 18 Every day – 16
Amount of alcohol considered safe/normal to drink during one evening (measured in shots of vodka or equivalent)	Between 1 and 2 – 182 Between 3 and 4 – 287 Between 5 and 6 – 206 Between 7 and 8 – 90 More than 10 – 49
Behavior considered indicative of having drunk too much alcohol	Self control impairment, sluggishness, verbosity – 353 Coordination and balance impairment, aggression – 307 Sleepiness, deep self control and balance impairment, mumbling, unsteady walk – 154
Past or current marijuana use (yes/no)	483/331
Frequency of marijuana use	Every day – 11 Few times a week – 25 Once a week – 12 Few times a month – 41 Less than once a month – 337

Table 3. Stages of alcohol intoxication based on [5]

Blood alcohol concentration	Exhibited behavior
Stage 1. 0.3 – 0.4 permille	Reduction of self-criticism; high mood and self-confidence. Longer reaction time and worsened coordination
Stage 2. 0.5 – 0.6 permille	Self control impairment, sluggishness, verbosity
Stage 3. 0.7 – 2.0 permille	Further coordination and balance impairment, aggression; lower concentration and balance; blood pressure and heart rate elevated
Stage 4. 2.0 – 3.0 permille	Sleepiness, deep self control and balance impairment, mumbling, unsteady walk
Stage 5. 3.0 – 4.0 permille	Deep consciousness impairment leading to coma; reflexes and all senses impaired; lowered blood pressure and body temperature; possible cardiac arrhythmia and breathing depression
Stage 6. over 4.0 permille	Coma, cardiac arrhythmia, breathing depression, blood pressure lowering

Subtle differences are evident in this particular aspect. About three times more men than women drink 3–4 times a week as well as residents of cities compared to those from villages. The distinction is even more noticeable in regard to drinking every day. Majority of students from both sexes drink less than once a week. Faculty chosen by students has less influence on their drinking habits than sex or original residence where those from the city are likely to drink more often than those from the village.

Table 5 shows how the amount of alcohol considered safe/normal to drink during one evening differs between the groups investigated.

Data show that sex related frequency of drinking corresponds with the amount of alcohol likely to be consumed by both sexes. Female students generally tend

Table 4. Frequency of alcohol consumption

Men	Less than once a week – 41.0% 1–2 times a week – 36.5% 3–4 times a week – 15.6% 5–6 times a week – 3.3% Every day – 3.6%
Women	Less than once a week – 65.7% 1–2 times a week – 26.6% 3–4 times a week – 5.1% 5–6 times a week – 1.6% Every day – 1%
Village	Less than once a week – 72.2% 1–2 times a week – 19.4% 3–4 times a week – 3.7% 5–6 times a week – 3.7% Every day – 0.9%
City	Less than once a week – 54.0% 1–2 times a week – 32.0% 3–4 times a week – 9.9% 5–6 times a week – 2.0% Every day – 2.1%
Medicine	Less than once a week – 58.3% 1–2 times a week – 29.4% 3–4 times a week – 7.5% 5–6 times a week – 2.5% Every day – 2.3%
Law	Less than once a week – 54.6% 1–2 times a week – 31.2% 3–4 times a week – 10.6% 5–6 times a week – 1.9% Every day – 1.7%

Table 5. Amount of alcohol considered safe/normal to drink during one evening (measured in shots of vodka or equivalent)

Men	Between 1 and 2 – 16.9% Between 3 and 4 – 29.6% Between 5 and 6 – 25.7% Between 7 and 8 – 16.3% More than 10 – 11.4%
Women	Between 1 and 2 – 25.6% Between 3 and 4 – 38.7% Between 5 and 6 – 25.0% Between 7 and 8 – 7.9% More than 10 – 2.8%
Village	Between 1 and 2 – 25.0% Between 3 and 4 – 30.6% Between 5 and 6 – 25.0% Between 7 and 8 – 10.2% More than 10 – 9.3%
City	Between 1 and 2 – 22.0% Between 3 and 4 – 36.0% Between 5 and 6 – 25.5% Between 7 and 8 – 11.2% More than 10 – 5.5%%
Medicine	Between 1 and 2 – 27.1% Between 3 and 4 – 37.2% Between 5 and 6 – 20.9% Between 7 and 8 – 9.0% More than 10 – 5.8%
Law	Between 1 and 2 – 17.8% Between 3 and 4 – 33.4% Between 5 and 6 – 29.6% Between 7 and 8 – 13.0% More than 10 – 6.2%

to drink less than male students. Students from villages are however more likely to go to the extremes as twice as many of them would drink more than 10 shots of vodka than those from the city. As before, there is no major distinction between medicine and law students. However, medicine students are more likely to stop at 1 or 2 shots (27.1%) than law students (17.8%).

Table 6 shows what behavior is considered by students from the investigated groups as being indicative of having drunk too much alcohol.

This variable finally differentiates medicine and law students. 26% of the latter group would stop at stage 4 of alcohol intoxication scale, the highest presented in the questionnaire. Roughly one in ten medicine students would venture that far, with half of them stopping at stage 2 (lowest presented to choose from). Only 36.2% of law students would finish drinking that early. Half of women in the study would bring drinking to a halt at the lowest stage while only 30.6% men would stop that early. Almost twice as many male students (27%) are likely to drink up until stage 4 symptoms occur; only 14% of women would drink as far as that.

Table 7 reveals what percentage of students evaluated in the study have ever tried marijuana.

There is almost no difference between faculties and only slight one between sexes and different residencies. Only 52.9% of women have ever tried marijuana whereas 70% of men have ever had any association with the drug. As for the students from cities and villages those values are 61.5% and 45.4%, respectively. Additional distribution of the answers between male and female students from both faculties who admitted to smoking cannabis was made (**Table 8**) showing the frequency of their marijuana use. Most of those who ever tried marijuana do not do it regularly but a relatively high percentage (between 10% and 37% depending on sex and faculty) do it at least few times a month, male law students being the most frequent smokers.

It should be noted, however, that this representation might not be accurate as the group of smokers within the study was low to begin with. Dividing it by frequency of use further dilutes the number of individuals amounting for each % point.

Table 6. Behavior considered indicative of having drunk too much alcohol

Men	Self control impairment, sluggishness, verbosity – 30.6% Coordination and balance impairment, aggression – 42.3% Sleepiness, deep self control and balance impairment, mumbling, unsteady walk – 27.0%
Women	Self control impairment, sluggishness, verbosity – 51.1% Coordination and balance impairment, aggression – 34.9% Sleepiness, deep self control and balance impairment, mumbling, unsteady walk – 14.0%
Village	Self control impairment, sluggishness, verbosity – 44.4% Coordination and balance impairment, aggression – 34.3% Sleepiness, deep self control and balance impairment, mumbling, unsteady walk – 21.3%
City	Self control impairment, sluggishness, verbosity – 43.2% Coordination and balance impairment, aggression – 38.2% Sleepiness, deep self control and balance impairment, mumbling, unsteady walk – 18.6%
Medicine	Self control impairment, sluggishness, verbosity – 50.8% Coordination and balance impairment, aggression – 37.7% Sleepiness, deep self control and balance impairment, mumbling, unsteady walk – 11.6%
Law	Self control impairment, sluggishness, verbosity – 36.2% Coordination and balance impairment, aggression – 37.7% Sleepiness, deep self control and balance impairment, mumbling, unsteady walk – 26.0%

Table 7. Past or current marijuana use

Men		Yes – 70.0% No – 30.0%
Women		Yes – 52.9% No – 47.1%
Village		Yes – 45.4% No – 54.6%
City		Yes – 61.5% No – 38.5%
Medicine	Male students	Yes – 69.3% No – 30.7%
	Female students	Yes – 55.2% No – 44.8%
Law	Male students	Yes – 70.7% No – 29.3%
	Female students	Yes – 50.6% No – 49.4%

Table 8. Frequency of marijuana use among male and female students

		Every day	Few times a week	Once a week	Few times a month	Less than once a month
Medicine	Male	1.0%	8.0%	3.0%	8.0%	80.0%
	Female	2.3%	1.5%	0%	6.2%	90.0%
Law	Male	4.6%	10.2%	7.4%	14.8%	63.0%
	Female	1.6%	3.1%	0.8%	7.0%	87.5%

Discussion

Mean age of alcohol initiation is very similar among students from all investigated groups. Therefore sex, residence and future faculty do not have any influence on further differences in alcohol drinking behavior (frequency and volume of liquor consumption and their manners after "hitting the bottle"). As young age of alcohol initiation is a risk factor for future binge drinking [6] it should be noted that individuals from all groups are equally susceptible to this major health concern.

More male than female physicians report hazardous (large amounts and/or frequent) drinking [7] and the study clearly shows that this tendency starts at least as early as during medical studies. However, there is a higher percentage of female doctors presenting hazardous drinking than women in general population. A lower percentage of hazardous drinkers is exhibited among male doctors than men in general population

[7]. The same concurrence is exhibited in regard to marijuana use among female and male doctors (higher in the latter group) as well as female and male students (**Table 8**). Additionally, higher illegal drug use is reported among doctors than in general population [7] which is concerning, as the group meant to educate and set an example for others is using more than those they should supervise and educate.

As apparent as the tendency among all students to drink excessively is from this study, it is not as observable in real life. One of the reasons may be the fact that students with drinking problems are more likely to seek help in their peers and parents [8] than any institutions able to reliably measure the magnitude of the problem. Drinking problems among students remain therefore not only mostly unsolved but, more importantly, unidentified.

The fact that medicine students are more likely to recognize too high alcohol intoxication (**Table 6**) than their peers from law school may be comforting, but the percentage of alcohol over-users among physicians suggests that this knowledge does not help them reduce the magnitude of the problem in their own behavior.

Preventive measures should be taken at an age as young as possible as cannabis users are more prone to failing their studies [9]. Additionally, marijuana use is known to have a negative influence not only on physical but mental health [5] which may in turn lead to lower competence of doctors exposed to drugs, not to mention legal problems that may significantly harm their career.

Conclusions

More emphasis should be put on educating both medical students and general public about dangers associated with hazardous drinking and drug use. As doctors are often the first to respond to their patients exhibiting signs of substance over-use, they should be competent enough not only to help others but also control and avoid being exposed to the problem themselves. It is especially important regarding the fact that quarter of deaths in young people is related to alcohol use.

Acknowledgements

Conflict of interest statement
The authors declare that there is no conflict of interest in the authorship or publication of contribution.

Funding sources
There are no sources of funding to declare.

References

1. Zadarko-Domaradzka M, Zadarko E, Barabasz Z, Sobolewski M. Alcohol use and health-risk behaviours among academic students in Podkarpackie. Przegl Lek. 2013;70(8):546–50.
2. Frank E, Rothenberg R, Lewis C, Belodoff BF. Correlates of physicians prevention-related practices. Findings from the Women Physicians. Health Study. Arch Fam Med. 2000;9:359–367.
3. Ham LS, Hope DA. College students and problematic drinking: A review of the literature. Clin Psychol Rev. 2003;23:719–759.
4. Anderson P. Global use of alcohol, drugs and tobacco. Drug Alcohol Rev. 2006;25(6):489–502.
5. Klimkiewicz A. Ostre zatrucie alkoholem. http://gastrologia.mp.pl/zatrucia/show.html?id=81259 (accessed 15.05.2015).
6. Elisaus P, Williams G, Bourke M, Clough G, Harrison A, Verma A. Factors associated with the prevalence of adolescent binge drinking in the urban areas of Greater Manchester. Eur J Public Health. 2015 Oct 1. pii: ckv115. [Epub ahead of print].
7. Voigt K, Twork S, Mittag D, Göbel A, Voigt R, Klewer J, Kugler J, Bornstein SR, Bergmann A. Consumption of alcohol, cigarettes and illegal substances among physicians and medical students in Brandenburg and Saxony (Germany). BMC Health Serv Res. 2009 Dec 3;9:219.
8. Oster-Aaland L, Lewis MA, Neighbors C, Vangsness J, Larimer ME. Alcohol poisoning among college students turning 21: do they recognize the symptoms and how do they help? J Stud Alcohol Drugs Suppl. 2009 Jul;(16):122–30.
9. Gignon M, Havet E, Ammirati C, Traullé S, Manaouil C, Balcaen T, Loas G, Dubois G, Ganry O. Alcohol, cigarette, and illegal substance consumption among medical students: a cross-sectional survey. Workplace Health Saf. 2015 Feb;63(2):54–63.

Acceptance for editing: 2015-02-10
Acceptance for publication: 2015-03-30

Correspondence address:
Wiktor Suchy
os. Lotnictwa Polskiego 16/50
60-406 Poznań, Poland
phone: +48 668 667 127
email: wiktor.suchy@icloud.com

ORIGINAL PAPER

DOI: https://doi.org/10.20883/medical.e16

Identifying patients' criteria for assessment of doctors on Polish physician rating websites

Radosław Tymiński, Michał Walczewski, Michał Wieczorek

Medical University of Warsaw

ABSTRACT

Introduction. Increasingly popular physician ranking websites have lately become a significant factor in choosing a physician.
Aim. The aim of this study was to establish the criteria by which patients assessed doctors on PRWs and which of these criteria were the most crucial during the general assessment of the physicians.
Material and methods. Selected narrative comments from two Polish PRWs: znanylekarz.pl and rankinglekarzy.pl were analysed on the basis of the following criteria: kindness and propriety, punctuality, communication with patients, condition and equipment of a doctor's office, length of the appointment, cost of the medical advice.
Results. Out of 4375 eligible comments kindness and propriety was assessed most frequently (3012 comments, 68.85%), next was communication, which was evaluated in 2343 comments (53.55%). Amongst the 3012 comments with assessed kindness and propriety, 77.66% (2339 comments) were described positively. In the group of comments with positively evaluated kindness and propriety 2230 comments (95.34 %) were generally positive. Furthermore, communication with patient was assessed in 2343 comments and in 1827 cases (77.98%) the assessment was positive. 1810 comments with positively evaluated communication were generally positive (99.07%).
Conclusions. There is a connection between the patients' positive assessment and physician's kindness, personal culture and communication skills; if physicians focus on the aforementioned abilities, it might lead to better physician perception, higher effectiveness of treatment and the lower number of potential law suits.

Keywords: physician ranking sites, doctor assessment, patient satisfaction, soft skills.

Introduction

As of late, physician rating websites (PRWs) are becoming easily accessible and increasingly popular [1] as sources of information and comments on doctors and other health professionals [2, 3]. The number of assessed doctors is increasing [4], and in contrary to the anxiety expressed by the medical profession, the majority of comments is unambiguously positive [1, 5–7]. Moreover, various studies indicate that for many patients PRWs have become a significant factor in choosing a physician [8]. Likewise, similar changes can be noticed in Poland, where the Internet is starting to play an important part as a source of information on health, diseases, treatment, as well as the assistance in choosing a physician [9].

On the basis of the results of previous studies it can be assumed that the ability to establish a good relationship with a patient is significant [6]. In our opinion, although this thesis is true, it insufficiently describes dependencies between assessment of a doctor and his or her personality traits and communication skills. We state that both politeness and propriety as well as the skills in interpersonal communication are essential components of a good doctor-patient relationship. Proper relations between both sides have influence not only on patient's compliance [10] and treatment results, but also on how a physician is perceived. In other words, we assumed the hypothesis that general patients'

assessment of doctors depends on the physicians' ability to build a relationship with patient.

The purpose of this study was to verify two important matters. The first aim of this study was to verify what exact features of a doctor or a medical service, apart from professional competence, determine if a patient's comment on physician is negative or positive in general.

Secondly, we wanted to establish whether the obligation imposed by the Polish legislator in Article 31 Paragraph 1 of the Medical Profession Act of 5 December 1996 to provide a patient with full medical information is justified by patients' actual expectations. Due to these regulations a doctor should inform a patient about: his or her state of health, diagnosis, proposed and available diagnostic and/or therapeutic methods, predictable results of application or abandonment of these methods, treatment results and prognosis.

In addition, our study should help identify areas where doctor-patient relationship difficulties are still persistent.

The accurate assessment of existing problems is going to be a first step in search of solutions, which may increase patient compliance, improve treatment results and consequently increase patients' subjective satisfaction.

Material and methods

Narrative comments from two Polish PRWs were selected: ***znanylekarz.pl*** and ***rankinglekarzy.pl***.

The analysed comments consisted of:

- comments on internists and pediatricians retrieved from znanylekarz.pl;
- comments on internists and gynaecologists retrieved from rankinglekarzy.pl;

Two different PRWs and a number of physicians with different medical specialties (internal medicine, gynaecology, pediatrics) were chosen to verify whether the results for each PRW and each medical specialty were comparable. If the results could be confirmed, it would suggest they are not dependent on the medical specialty nor the PRW.

Because of the large number of comments on each group of medical specialists and our intention to analyse every comment in each group on both PRWs, the study was limited to the physicians (from public and private healthcare) who practice medicine in Warsaw and its neighborhoods.

The comments from doctor profiles were included based on the following conditions:

1. the doctor's profile appeared in PRW's search engine results after typing in (in Polish) phrases: "gynaecologist Warsaw", "Warsaw gynaecologist", "internist Warsaw", "Warsaw internist", "pediatrist Warsaw", "Warsaw pediatrist";
   and
2. at least 10 comments were assigned to the doctor's profile by a website administrator.

Two of the authors independently analysed all the narrative comments from the doctor profiles which met the aforementioned requirements, provided the comment satisfied at least one criterion of comments analysis (**CNCA**, see "The criteria of narrative comments analysis").

Therefore the comments from the following doctor profiles were excluded from the study:

- the profile did not appear in search results after typing in the phrases mentioned above.
- the profile had less than 10 comments assigned. We resigned from the analysis of these comments in order to exclude random comments, those which seemed to be emotionally biased, those written by the physicians themselves, on their request or by their competitors.

After classification, the comments were reassessed and those not meeting the CNCA were rejected.

The excluded comments can be divided into three main groups:

1. **comments containing technical data –** these comments did not contain any assessment of a physician; they referred mainly to the issues like physician work hours, his or her current workplace or a phone number.
2. **comments referring only to professional competence of the physician –** these comments contained only the assessment of professional competence, including accuracy of diagnostic process and treatment. Comments made by patients assumed to have been unable to assess this sphere properly, due to the task being outside of their competence, were disqualified.
3. **very brief comments** – comments which did not contain any justification for included assessment.

The qualification process was presented on the **Figure 1**.

All the comments qualified were analysed on the basis of **the criteria of narrative comments analysis (CNCA**), which had been developed after the initial analysis of 80 comments. We established the following criteria of comment analysis:

1. **Kindness and propriety**

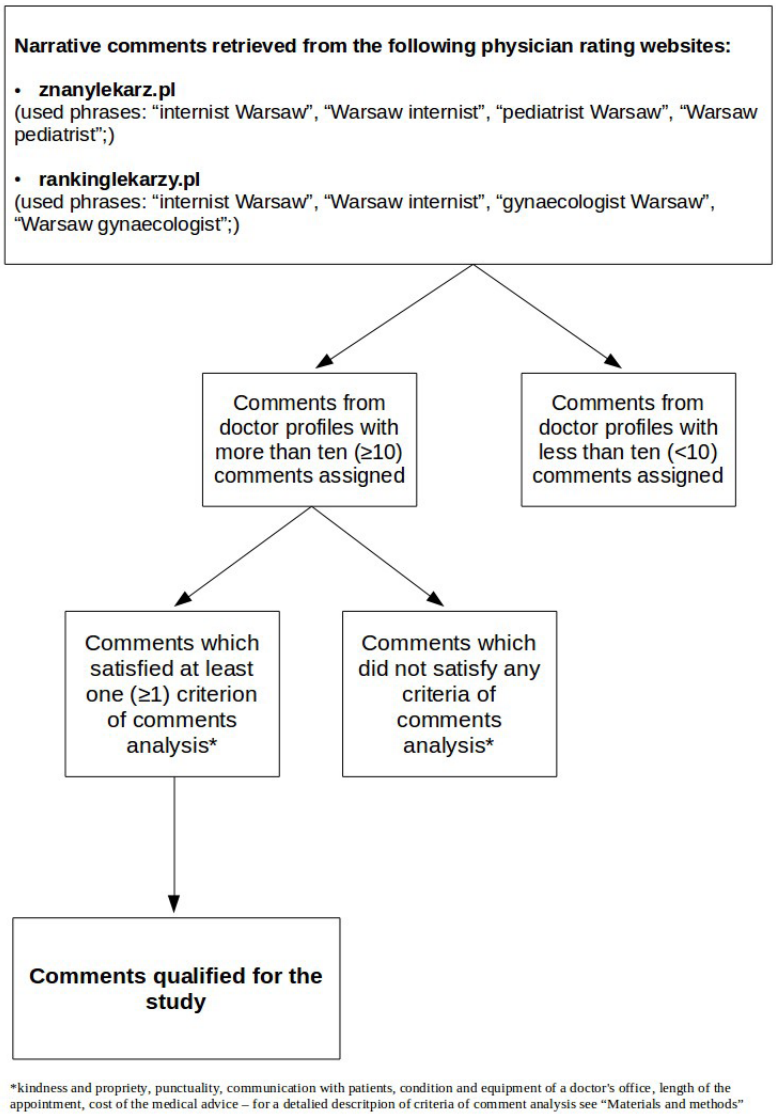

Figure 1. Qualification process

(Was the physician kind, polite, well-mannered, friendly, obliging, gentle, considerate?)

2. **Punctuality**
(Did the physician arrive at the consulting room on time?)
3. **Communication with patients**
(We assumed that communication was assessed in two situations: when the patient expressed his opinion on the **general** verbal and non-verbal communication skills of the physician [these were mostly the comments in which the patient stated whether physician talked with the patient, maintained eye contact] or when the patient described **in depth** doctor's communication skills, dividing them into one or more components [listening, explaining, asking questions]; in this case we assumed that the patient assessed general communication as well as in-depth communication).

3a. **Listening**
(Did the physician listen carefully to the patient?)

3b. **Explaining**
(Did the physician of their own will explain to the patient the pathophysiology of the disease? Did the doctor provide the patient with intelligible information about his or her state of health, diagnosis, proposed and available diagnostic and/or therapeutic methods, predictable results of application or abandonment of these methods, treatment results and prognosis.)

3c. **Asking questions**
(Did the patient have an opportunity to ask questions during the medical appointment? Did the physician answer those question?)

4. **Condition and equipment of a doctor's office**
   (Was the doctor's office clean? Was the equipment of good quality?)
5. **Length of the appointment**
   (Was the patient satisfied with the length of the medical appointment?)
6. **Cost of the medical advice**
   (Did the patient regard the cost of the medical advice as appropriate?)

Every qualified comment was analysed on the basis of the criteria mentioned above. If justification for the assessment which met the specific CNCA was found in a specific comment, then that assessment was qualified as positive or negative within a particular CNCA. It meant that the described by the specific CNCA aspect of medical practice was assessed respectively positively or negatively by the patient.

In our study we assumed that every comment could be qualified as:

- generally **positive** (a doctor who was assessed as very good or good on znanylekarz.pl or a doctor who received between 3,5/5 and 5/5 stars (⩽ 5 and > 3,5 stars) on rankinglekarzy.pl);
- generally **negative** (a doctor who was assessed as weak or very weak on znanylekarz.pl or a doctor who received between 1/5 and 2,5/5 stars (⩾ 1 and < 2,5 stars) on rankinglekarzy.pl);
- generally **neutral** (a doctor who was assessed as neutral on znanylekarz.pl or a doctor who received between 2,5/5 and 3,5/5 stars (⩽ 3,5 and ⩾ 2,5 stars) on rankinglekarzy.pl).

If the comment was qualified to one of groups above, it meant that the general assessment of a physician in this comment was respectively positive, negative or neutral. As a result, the relation between the CNCA and a patient's general assessment of a doctor could be verified.

Results

From December 2014 to January 2015 4 groups of narrative comments were initially analysed: 2616 comments on 142 gynecologists and 560 comments on 36 internists from PRW rankinglekarzy.pl and also 2192 comments on 113 internists and 2321 comments on 121 pediatricians from PRW znanylekarz.pl . Altogether 4375 (56,90%) comments met the criteria described in Methodology part and were included in the final analysis.

Out of 4375 eligible comments 3294 (75,39%) were generally positive, 1002 (22,90%) were negative and only 79 (1,81%) were neutral.

Every narrative comment was analysed according to CNCA described in Methodology. Amongst all 4375 eligible comments most (3012 comments, 68,85%) assessed kindness and propriety. Communication was evaluated in 2343 comments (53,55%). Another most frequently assessed aspect of medical appointment was its length which was mentioned in 317 comments (7,25%). It is necessary to emphasise that 759 (41,93%) out of 1810 comments regarding communication in general were focused on a physician's ability to explain important matters concerning medical con-

Table 1. Results of particular categories divided into generally positive, negative and neutral comments

Assessed element	Number of concerns						Total
	Generally positive comments (n = 3294)		Generally negative comments (n = 1002)		Generally neutral comments (n = 79)		
	Assessed positively	Assessed negatively	Assessed positively	Assessed negatively	Assessed positively	Assessed negatively	
Kindness and propriety	2230	29	75	630	34	14	3012
Punctuality	12	7	0	30	0	2	51
Communication with patient (listening, explaining, asking questions)	1810	23	7	462	10	31	2343
Listening	337	1	5	61	3	3	410
Explaining	759	6	1	102	2	8	878
Asking questions	491	2	2	124	2	3	624
Condition and equipment of the doctor's office	18	1	1	6	1	0	27
Length of the appointment	173	8	0	125	0	11	317
Cost of the medical advice	21	14	0	32	0	6	73
Total	5851	91	91	1572	52	78	7735

ditions. They also mentioned the course of the appointment (**Table 1**).

In comments regarding internists and gynecologists, patients most often assessed kindness and propriety, then communication skills and then length of the appointment. By contrast, in comments concerning pediatricians communication with patient was the most frequently assessed aspect of the appointment, followed by kindness and propriety, and then length of the visit (**Table 2**).

Amongst the 3012 comments with assessed kindness and propriety, 77,66% (2339 comments) were described positively. In the group of comments with positively evaluated kindness and propriety 2230 comments (95,34 %) were generally positive. Furthermore, communication with patient was assessed in 2343 comments and in 1827 cases (77,98%) the assessment was positive. 1810 comments with positively evaluated communication were generally positive (99,07%) (**Figure 2**).

Discussion

Present literature

Despite the growing popularity of PRWs amongst patients it appears that the researchers' interest in this subject is modest. On 15.10.2014 we searched the PubMed database using phrases „physician rating sites", „rating sites", „physician rating websites" and received 11, 20 and 23 search results respectively. In the Cochrane Library no results were found. Publications in the PubMed database focused e.g. on socio-

Table 2. Assessment of kindness and propriety and communication in different medical specialties. Generally neutral comments not included

Assessed element	Generally positive comments		Generally negative comments		Total
	Assessed positively	Assessed negatively	Assessed positively	Assessed negatively	
Gynecologists, rankinglekarzy.pl					
Kindness and propriety	777	14	39	334	1164
Communication with patient	579	8	4	205	796
Internists, rankinglekarzy.pl					
Kindness and propriety	158	0	1	74	233
Communication with patient	103	0	0	47	150
Internists, znanylekarz.pl					
Kindness and propriety	722	6	17	85	830
Communication with patient	507	9	1	80	597
Pediatricians, znanylekarz.pl					
Kindness and propriety	573	9	18	137	737
Communication with patient	621	6	2	130	759

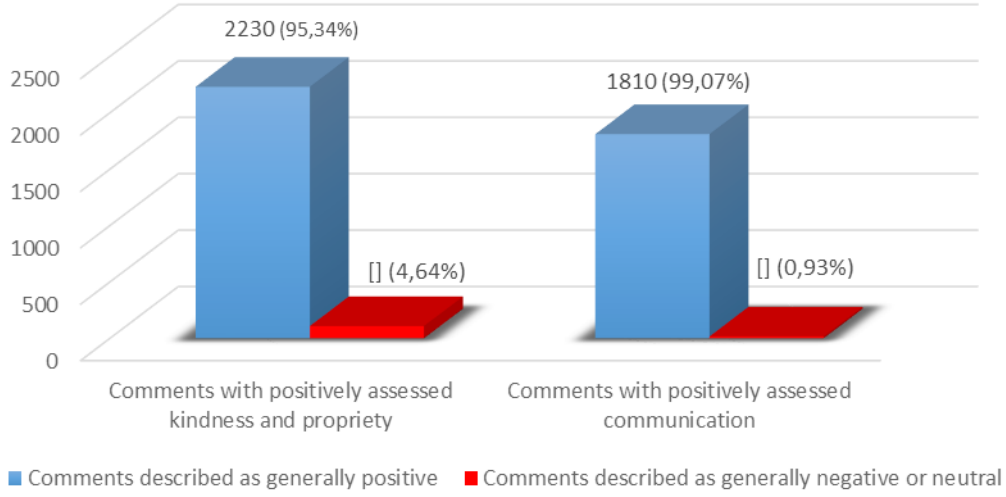

Figure 2. Dependency between positive assessment of communication or kindness and the general evaluation of the doctor

demographic analysis of PRW's users [24] or on the analysis of physicians' rating criteria on different websites [12]. Most of the studies we found originate from Germany, the United Kingdom and the United States of America. The majority of available literature was focused on scaled survey rating results [31, 32] and only few research papers concentrated on narrative comments analysis.

Studies focused on narrative comments analysis

During further online search, 5 studies concerning narrative comments analysis were retrieved. In the paper published in October 2014, Emmert et al. analysed 3 000 narrative comments about German general practitioners and specialists [25]. Authors investigated 1500 randomly selected narrative comments on general practitioners and 1500 comments on specialists posted on the German PRS – jameda. Researchers evaluated patients' concerns mentioned in narrative comments and classified them into 50 different sub-categories from 3 main categories: physician, office staff, and practice. It is worth mentioning that the patient's perception of the physician's professional competence was included in the analysis.

The main goal of the American study conducted by Lopez et al. was to list all elements concerning medical appointment found during analysis of 712 randomly selected narrative comments posted on PRWs [26]. Greaves et al. and Alemi et al. evaluated American and British patients' online opinions using the artificial intelligence methods [27, 28]. Lagu and colleagues analysed patients' text comments on hospitals posted in the first government-sponsored healthcare rating website – NHS Choices [29].

Majority of comments are positive

The results of our study are consistent with earlier research and confirms the previous conclusion that most comments published on PRWs are positive [1, 5–7, 12, 25]. Our data presents that 77,39% from all eligible comments were positive. This particular observation is important in ongoing discussion with opponents of PRWs who claim, that physician rating websites are perfect platforms for defamation of doctors [12]. Interestingly enough, our results show that minority of comments are neutral (1,81%) and these findings are consistent with the study of Emmert et al. [25]. That leads as to an important conclusion that physicians are assessed more frequently when patients are significantly satisfied, or on the contrary – significantly dissatisfied.

Most frequently assessed aspects

Outcomes of our study indicate, that regardless of doctor's specialization most of patients' concerns focus on: physician's kindness and propriety, communication with patient, length of the appointment. Those concerns occurred in 66,85%, 53,55% and 7,25% of all eligible comments respectively. According to Emmert et al. patients in PRWs most frequently describe physician's professional competence, then friendliness and caring attitude, and then time spent with the patient [25]. Information and communication are on 4th and 7th position in the list accordingly. As we can observe the main difference between our study and the German research lies in including patients' perception of physician's competence in the analysis. Presumably, other discrepancies in frequency of particular patient's concerns results from two issues. At first, it can be caused by differences between Polish and German patients. Secondly, our category "Communication with patient" is a combined point for plenty of aspects connected with communication (as described in "Methodology"). On the contrary, in the German study those aspects are separated in many different subcategories e.g. being taken seriously by a doctor or physician's child-friendliness.

Furthermore, analysis of differences in results between medical specialties demonstrates that pediatricians' communication with patients was evaluated more often than their kindness and propriety (**Table 2**). This discrepancy can be caused by the fact, that PRW users which comment on physicians from this exact specialty are mostly parents who pay heed to physician's communication with children and clear explanation of their child's medical condition.

Key factor – communication

As indicated before, patients in narrative comments posted in PRWs most frequently described kindness and propriety (66,85%) which in 77,66% of cases was assessed positively. If kindness and propriety was assessed positively, 95,34% of comments were generally positive (**Figure 1**). For communication this dependency was 99,07%. In other words if physician's communication with a patient was assessed positively the predominant majority of comments were generally positive. These findings are substantial in the light of research signifying that proper doctor-patient communication results in improved patient's satisfaction from contact with a medical professional [22, 30]. Fong Ha and Longnecker in their systematic review of literature pointed out studies which proved increased satisfac-

tion from the medical appointment, abridged hospitalization, quicker convalescence, decreased pain sensation and better adherence to treatment in patients with good communication with a physician [30]. Bearing in mind this information, it is worth mentioning that public opinion poll carried out in 2014 indicates that only 54% of Polish people consider that patients in national healthcare system are treated with kindness [16].

Significance of explanation

Outcomes of our analysis demonstrate that amongst 1810 narrative comments concerning communication, most of the comments (41,93%) were related to physician's willing explanation of diseases' patomechanisms and providing complete information about patient's medical condition, possible therapeutic options and their adverse effects (**Table 1**). Also in the German study cited above, information was the most frequently assessed subcategory related to communication [25]. Survey carried out amongst parents, whose children suffered from brachial plexus palsy during childbirth shows that poor communication and incomplete information correlated with more frequent malpractice litigations [17]. Moreover, previous research indicated that physicians who were sued more often, tended to get more complaints concerning their communication with patient [18, 19].

Limitations of the study

Major limitation of the study is that it concerns only online comments which are posted by a specific group of patients that uses PRWs and cannot perfectly represent the general population. Moreover we only addressed comments on 3 medical specialties and it is possible that there are significant differences in other specialties. Moreover we analysed comments from two major polish PRWs therefore there is a possibility that analysis of other websites can bring divergent results. Further research on this field are needed.

Conclusion

The conducted study reached designated goals. Although the study results cannot be completely extrapolated to the general patients' assessment of doctors, we claim that nevertheless the conclusions based on the studied PRWs are significant. Firstly it should be noticed that the majority of comments, which can be found on the studied PRWs, are positive and only few of them are neutral. Therefore PRWs can not be completely trusted as the source of information on a particular physician, because the majority of patients, who have a broadly defined neutral opinion and are neither very satisfied nor disappointed with the doctor, probably do not use PRWs.

The most important achievement is that, in our opinion, we managed to prove a connection between the patients' positive assessment and physician's kindness, personal culture and communication skills. We believe that this conclusion is significant, because it clearly indicates that the positive assessment of a doctor depends on his or her relationship with a patient. Moreover it implies that physicians should attach more importance to socio-psychological aspects of their practice and therefore the development of soft skills should be a vital part of medical training. In addition, our study demonstrated that, in many comments, parents referred to the importance of a good relationship between a doctor and a child; this matter should be particularly important to pediatricians.

We proved that, for patients, the most important parts of communication with a doctor were detailed explanation and providing full medical information; it should be particularly noted that this conclusion is completely consistent with current legal situation, which requires doctors to provide a patient with full medical information.

Our findings allow us to present a hypothesis that there is a connection between the constantly increasing number of lawsuits against physicians based on the presumed or actual violation of patients' right to information, and the factual lack of full medical information in certain cases.

To sum up, kindness, politeness, and good communication with a patient might lead to better physician perception, higher effectiveness of treatment and the lower number of potential law suits. [17–19, 30].

Acknowledgements

The authors would like to thank Mr Jarosław Woś for language support.

Conflict of interest statement
The authors declare that there is no conflict of interest in the authorship or publication of contribution.

Funding sources
There are no sources of funding to declare.

References

1. Gao GG et al. A Changing Landscape of Physician Quality Reporting: Analysis of Patients' Online Ratings of Their Physicians Over a 5-Year Period. Journal of Medical Internet Research. 2012;14(1):e38.

2. Verhoef LM et al. Social Media and Rating Sites as Tools to Understanding Quality of Care: A Scoping Review. Journal of Medical Internet Research. 2014;16(2):e56.
3. Mostaghimi A, Crotty BH, Landon BE. The Availability and Nature of Physician Information on the Internet. Journal of General Internal Medicine. 2010;25(11):1152–1156.
4. Hanauer DA et al. Public awareness, perception, and use of online physician rating sites. Jama. 2014;311(7):734–5.
5. Emmert M, Meier F. An analysis of online evaluations on a physician rating website: evidence from a German public reporting instrument. J Med Internet Res. 2013;15(8):e157.
6. Lopez A et al. What patients say about their doctors online: a qualitative content analysis. J Gen Intern Med. 2012;27(6):685–92.
7. Kadry B et al. Analysis of 4999 online physician ratings indicates that most patients give physicians a favorable rating. J Med Internet Res. 2011;13(4):e95.
8. Emmert M et al. Physician choice making and characteristics associated with using physician-rating websites: cross-sectional study. J Med Internet Res. 2013;15(8):e187.
9. Polish Internet research. Online health websites. 2013.
10. Zolnierek KB, Dimatteo MR. Physician communication and patient adherence to treatment: a meta-analysis. Med Care. 2009;47(8):826–34.
11. Terlutter R, Bidmon S, Rottl J. Who uses physician-rating websites? Differences in sociodemographic variables, psychographic variables, and health status of users and nonusers of physician-rating websites. J Med Internet Res. 2014;16(3):e97.
12. Strech D, Reimann S. [German language physician rating sites]. Gesundheitswesen. 2012;74(8–9):e61–7.
13. Emmert M, Sander U, Pisch F. Eight questions about physician-rating websites: a systematic review. J Med Internet Res. 2013;15(2):e24.
14. Emmert M et al. What do patients say about their physicians? An analysis of 3000 narrative comments posted on a German physician rating website. Health Policy. 2014;118(1):66–73.
15. Greaves F et al. Use of sentiment analysis for capturing patient experience from free-text comments posted online. J Med Internet Res. 2013;15(11):e239.
16. Alemi F et al. Feasibility of real-time satisfaction surveys through automated analysis of patients' unstructured comments and sentiments. Qual Manag Health Care. 2012;21(1):9–19.
17. Lagu T et al. A mixed-methods analysis of patient reviews of hospital care in England: implications for public reporting of health care quality data in the United States. Jt Comm J Qual Patient Saf. 2013;39(1):7–15.
18. Levinson W, Hudak P, Tricco AC. A systematic review of surgeon-patient communication: strengths and opportunities for improvement. Patient Educ Couns. 2013;93(1):3–17.
19. Ha JF, Longnecker N. Doctor-Patient Communication: A Review. The Ochsner Journal. 2010;10(1):38–43.
20. Public Opinion Research Center, Opinions About Healthcare System. 2014.
21. Domino J et al. Lack of physician-patient communication as a key factor associated with malpractice litigation in neonatal brachial plexus palsy. J Neurosurg Pediatr. 2014;13(2):238–42.
22. Levinson W et al. Physician-patient communication. The relationship with malpractice claims among primary care physicians and surgeons. Jama. 1997;277(7):553–9.
23. Hickson GB et al. Obstetricians' prior malpractice experience and patients' satisfaction with care. Jama. 1994;272(20):1583–7.

Acceptance for editing: 2015-02-10
Acceptance for publication: 2015-03-30

Correspondence address:
Radosław Tymiński
9 Łukowska Str, flat 126
04-133 Warsaw, Poland

REVIEW PAPER

DOI: https://doi.org/10.20883/medical.e17

Hemorheological studies of chosen clinical cases

Anna Marcinkowska-Gapińska, Piotr Kowal

Rheological Lab, Department of Neurology, Poznan University of Medical Sciences, Poland

ABSTRACT

Rheology – the study of the flow of matter and accompanying phenomena of real bodies deformation – in relation to blood – hemorheology. Blood viscosity – the main rheological parameter – has been studied in many research centers and among many different group of patients. The main disorders related to the hemorheological properties are: coronary insufficiency, vascular congestion, myocardial infarction, cerebral circulation disorder, Reynaud disease, ischemic limbs, diabetes, anemia, tumors. The following parameters are the main blood viscosity determinants: plasma viscosity, hematocrit, red cell deformability and erythrocytes aggregation. In hemorheological studies we used mathematical rheological models. The measurements of blood and plasma viscosity are performed by means of oscillating-rotary rheometers in order to determine the dependence of blood viscosity on the shear rate and the two components of the complex blood viscosity. Determination of blood cells aggregability and deformability is performed directly by means of aggregometers and appropiate filters and indirectly using rheological techniques with advanced mathematical models of blood viscoelasticity. Blood and plasma viscosity are subject to autoregulation mechanisms of the body. Recognition of those mechanisms may help in assessment of some diseases risk: ischemic stroke or myocardial infarction. In many cases rheological measurements may reveal the most recent phases of diseases and disorderses which enables early therapy with specimens improving the blood fluidity. For this reason rheological measurements should be applied in diagnostics and therapy. Mutual relations between the main factors determining the blood viscosity and their effect on blood flow are the main subject of current report.

Keywords: hemorheology, blood viscosity, abnormal aggregability, abnormal deformation.

Introduction

Blood is a systemic liquid of clearly non-Newtonian character and of distinctly pronounced viscoelastic properties. Blood flow in the circulatory system depends on the physical and physicochemical properties of blood as well as on many phenomena resulting from the structure and properties of the circulatory system.

Blood is a suspension of morphotic blood elements (erythrocytes, leukocytes, blood platelets) in plasma and its viscosity is dependent on the shear rate. Analysis of the physico-chemical properties of blood is related to hemorheology – a field of science focusing on the phenomena related to the flow of blood. Blood viscosity is determined by the following parameters: plasma viscosity, hematocrit, red cell deformability and aggregability [1, 2]. Shear rate dependence of blood viscosity and the domination of aggregation and deformation phenomena in forming the shape of this dependence were illustrated in the famous experiment by Chien [1, 3]. Blood plasma is a Newtonian fluid, whose viscosity does not depend on the shear rate. Plasma viscosity is determined by the presence of high molecular weight proteins like fibrinogen, immunoglobulins and lipoproteins [4]. Blood and plasma viscosity are subject to direct regulation by the organism. In particular one finds regulation achieved by changing the hematocrit and plasma viscosity [2].

Rheological studies of blood properties including both rotary measurements (flow curve) and oscillatory experiments (Dynamic Mechanical Analysis – DMA) are often complemented by experiments directly measuring red cells aggregability and deformability [1,

5–7]. There are many techniques allowing achieving such results [5–8]. Similar information about erythrocytes properties can be achieved indirectly from the mathematical analysis of the flow curve with the use of rheological models [1, 9]. In the literature one can find many models describing the flow of fluids. In the case of the flow of blood one should mention the model of Quemada [1, 10, 11].

Aim

The aim of the present work was the analysis of the rheological properties of blood in a group of patients with different disorders on the basis of experimental results and available literature data.

Hemorheology of chosen clinical cases

The analysis of rheological properties of blood samples taken from patients with different disorders revealed not only different shape of obtained rheograms [12] but also statistically significant differences of rheological parameters values compared to the results in the reference group. The latter effect was observed in patients with cardiac disorders [9, 13], diabetes [13, 14], and neurological disorders [15–17].

Patients with acute ischemic stroke show elevated whole blood viscosity resulting from the increase in plasma viscosity (high fibrinogen concentration) and also from intensified erythrocytes aggregation and reduced red cells deformability. In patients after cerebral ischemic episode (a few months after the symptoms of ischemic stroke) no increase of blood viscosity is observed despite still elevated plasma viscosity, which probably results from some improvement of red cells elasticity. An inverse correlation between plasma viscosity and erythrocytes deformability in this group of patients has been found [16].

In a group of patients with clinically silent foci of cerebral ischemia no increase of whole blood or plasma viscosity have been found [18]. The improved red cells deformability observed in this group should probably be attributed to an autoregulatory mechanism. A negative feed-back between the IgM/fibrinogen indicator and erythrocytes aggregability has been found in this group which suggests an opposite role played by these two proteins in the aggregation phenomenon of red cells.

In patients with diabetes mellitus an elevated plasma viscosity is found which results in increased whole blood viscosity. In this group of patients intensified erythrocytes aggregability and reduced deformability have been found. Hemorheological factors probably play an important role in the formation of diabetic microangiopathy [19]. Analysis of the thermographic picture of upper and lower limbs perfusion and the hemorheological profile in diabetic patients revealed no statistically significant correlations. However, an increased value of whole blood viscosity was found in comparison to the control group as well as an increased tendency to pathological erythrocytes aggregation [13, 14].

Other examples are: patients with monoclonal paraproteinemia (Waldenstrom disease and multiple myeloma) – in these patients one observes increase of the blood viscosity as a result of elevated plasma viscosity which in turn results from excessive concentration of monoclonal proteins. Human organism regulates these rheological disorders by reduction of the synthesis of other immunoglobulins and erythropoietin (EPO). An inverse relation between plasma viscosity and EPO concentration has been found [20]. Plasma viscosity has been found as one of the factors influencing the transcription of the EPO gene, next to intracellular oxygen concentration and the HNF-4 factor (Haptocyte Nuclear Factor 4).

Changes of the values of hemorheological parameters were observed also in a group of patients with nephrotic syndrome in which a decrease of plasma proteins concentration occurs, mainly albumins. Albumins belong to low molecular weight proteins and their effect on plasma viscosity is connected with their high concentration in plasma. Regulatory response of the organism to a loss of albumins is an increased synthesis of fibrinogen and other macroglobulins. In nephrotic syndrome one also finds elevated concentration of lipids in plasma, which in turn is regulated by an inverse relation with the plasma viscosity [2].

Patients with true polycythemia show elevated blood viscosity as a result of high hematocrit value. These patients also demonstrate high plasma viscosity which results in a decrease of EPO concentration. Other harmful phenomena related to this pathology are reduced erythrocytes elasticity and an increased red cells aggregability.

In patients with arterial hypertension the increase of whole blood viscosity is a result of elevated hematocrit value and reduced red cells elasticity. In a group of patients with arterial hypertension after ischemic stroke a reduced activity of fibrinogen molecules in forming the inter-erythrocyte connections [21].

Therapeutic methods influencing the hemorheological parameters

The HELP system (heparin extracorporeal LDL precipitation), in which certain protein and lipid components of plasma are eliminated by means of specific filters, which allows for improvement of the hemorheological profile in the range of all parameters (blood viscosity, plasma viscosity, red cells aggregability and deformability) [22].

Classic plasmapheresis is a method in which a part of plasma is removed from the organism and then replaced with fusions of albumin suspensions or other blood substitutes. In this way a reduction of plasma and hence also whole blood viscosity is achieved (mainly in the range of small shear rates) as well as lowering of red cells aggregability [23]. Thefore, the hemorheological effect resembles the one observed in the case of using the HELP system.

Patients treated by intravenous infusions of gamma globulins. As a result of such therapy, plasma viscosity increases, but also the red cells elasticity improves which prevents increasing the whole blood viscosity. In the range of low shear rates the relative blood viscosity may even be reduced [24].

Hemodilution is a therapeutic method in which highly oncotic fluids are infused to the circulatory system, increasing in this way the blood volume and reducing the hematocrit value and blood viscosity. The use of hydroxyethyl starch (HES) additionally gives an effect of plasma viscosity reduction (due to low molecular weight of starch) as well as reduction of red cells aggregability; moreover the dilution effect last longest [25]. On the contrary, the use of low molecular dextrans does not provide such a positive influence on the hemorheological properties and even may have pro-aggregative function [25].

Conclusions

In this work we have presented the effect of the changes in hemoreological properties of blood in chosen clinical states or being a result of undertaken therapy. The analysis of changes of physico-chemical properties of blood performed in this work shows how important the hemorheological factor can be in applied therapy. Many hemorheological aspects of living organism functioning are not known yet. We believe that thanks to the new research methods we should learn better the autoregulatory mechanisms aiming at improvement of blood flow in order to be able to utilize them in the case when a living organism cannot do it by itself.

Acknowledgements

KBN nr N N402 482037

Conflict of interest statement
The authors declare that there is no conflict of interest in the authorship or publication of contribution.

Funding sources
There are no sources of funding to declare.

References

1. Lerche D, Bämler H, Kucera W, Meier W, Paulitschke M. Flow properties of blood and hemorheological methods of quantification. In: Physical Characterization of Biological cells. Basic research and clinic relevance. Scütt W, Klinkmann H, Lamprecht I, Wilson T (eds.). Verlag Gesundheit GmbH Berlin, 1991; 189–214.
2. Reinhart WH. Molecular biology and self-regulatory mechanism of blood viscosity. A review. Biorheology. 2000;38:203–212.
3. Chien S, Jan KM. Ultrastructural basis of the mechanism of rouleaux formation. Microvas Res. 1973;5:155–166.
4. Lerche D, Koch B, Vlastos G. Flow behaviour of blood. Rheology. 1993;93:105–112.
5. Hardeman MR, Goedhart PT, Schut NH. Laser assisted Optical Rotational Cell Analyser (L.O.R.C.A). Red blood cell deformabitlyty; Elongation index versus cell transit time. Clin Hemorheol. 1994;14:619–630.
6. Hardeman MR, Goedhart PT, Dobbe JGG. Laser assisted Optical Rotational Cell Analyser (L.O.R.C.A). A new instrument for measurement of variuous structural hemorheological parameters. Clin. Hemorheol. 1994;14:605–618.
7. Sandhagen B. Assesment of blood rheology. Methodology and studies in healthy individuals, in patients with certain diseases and during liquid blood preservation. Acta Universitatis Upsaliensis, Uppsala; 1988.
8. Musielak M. Red blood cell-deformability measurenent: review of techniques. Clin Hemorheol Microcirc. 2009; 42:47–64.
9. Marcinkowska-Gapińska A, Gapiński J, Elikowski W, Jaroszyk F, Kubisz L. Comparison of three rheological models of shear flow behaviour studied on blood samples from post-infarction patients. Med Biol Eng Comp. 2007;45: 837–844.
10. Quemada D. A rheological model for studying the hematocrit dependence of red cell – red cell and red cell – and red cell – protein interactions in blood. Biorheology. 1981;18:501–516.
11. Quemada D. Blood rheology and its implication in flow of blood. In: Arteries and arterial blood flow. Rodkiewicz CM (ed.). Springer Verlag, Vien-New York, 1983; 1–127.
12. Marcinkowska-Gapińska A, Jaroszyk F, Kubisz L. Blood rheograms made on patients after myocardial infarction. Sci. Proc. Riga Tech. Univ. Ser. 6. Transport and Engineering, 2002; 138–142.
13. Marcinkowska-Gapińska A, Kowal P. Blood fluidity and thermography in patients with diabetes mellitus and

coronary artery disease in comparison to healthy subjects. Clin Hemorheol Microcirc. 2006;35:473–479.
14. Kowal P, Marcinkowska-Gapińska A. Badanie korelacji obrazu termograficznego z profilem hemoreologicznym u osób chorych na cukrzycę. Neuroskop. 2004;6:128–131 (in Polish).
15. Kowal P, Marcinkowska-Gapińska A. Comparison of the hemorheological parameters of blood in the groups of patients after cerebral stroke and myocardial infarction. Phys Med. 2004;20(Supplement):105–107.
16. Kowal P, Marcinkowska-Gapińska A. Hemorheological changes dependent on the time form the onset of ischemic stroke. J Neurol Sci. 2007;258(1–2):132–136.
17. Marcinkowska-Gapińska A, Kowal P. Comparative analysis of chosen hemorheological methods in a group of stroke patients. Clin Hemorheol Microcir. 2009;41:27–33.
18. Kowal P, Siemieniak I, Marcinkowska-Gapińska A. Próba oceny zmian hemoreologicznych w grupie pacjentów z niemymi klinicznie ogniskami niedokrwienia mózgu. Neuroskop. 2009;11:41–43 (in Polish).
19. Cho YI, Money MP, Cho DJ. Hemorheological disorders in diabetes mellitus. J Diabetes Sci Technol. 2008;2:1130–1238.
20. Singh A, Eckardt KV, Zimmermann A, Gotz KH, Hamann M, Ratcliffe PJ, Kurtz A, Reinhart WH. Increased plasma viscosity as a reason for inappropriate erythroprotein formation. J Clin Invest. 1993;91:251–256.
21. Kowal P. Arterial hypertension decreases fibrinogen molecules contribution to the inter-red cells connections in stroke patients. Clin Hemorheol Microcir. 1999;21: 321–324.
22. Seidel D. The HELP system: an efficient and safe method of plasmatherapy in the treatment of severe hybercholesterolemia. Ther Umsch. 1990;47:514–519.
23. Kowal P, Marcinkowska-Gapińska A, Kędzierski A, Siemienia I, Czekalski S, Kozubski W. Wpływ plazmaferezy klasycznej na profil hemoreologiczny u pacjentów z chorobami układu nerwowego. Badania pilotażowe. Neuroskop. 2009;11:34–36 (in Polish).
24. Kowal P, Zmyślony A. Hemorheological changes after intravenous gammaglobulin administration in patients with neurological disorders. Clin Hemorheol Microcir. 2008;40:229–234.
25. Marcinkowska-Gapińska A, Kowal P, Chałupka Z. The changes of low-shear rate hemorheological properties depending on the fluid used for transfusion. Clin Hemorheol Microcir. 2002;27:171–176.

Acceptance for editing: 2015-02-10
Acceptance for publication: 2015-03-30

Correspondence address:
Anna Marcinkowska-Gapińska
Rheological Lab, Department of Neurology,
Poznan University of Medical Sciences, Poland
49 Przybyszewskiego Str., 60-355 Poznan, Poland
fax: +48 (61) 8691 697,
email: margap@ump.edu.pl

REVIEW PAPER

DOI: https://doi.org/10.20883/medical.e18

Thin basement membrane disease – literature review

Jakub Żurawski

Chair of Biology and Environmental Protection, Department of Immunobiochemistry
Poznan University of Medical Sciences, Poland

ABSTRACT
Initially, the thin glomerular basement membrane disease was called "a gentle and curable hemorrhagic nephritis". The thin basement membrane disease has been finally characterized at the beginning of 1970s. This is when the connection between previously clinically described gentle microhematuria and significant thinning of glomerular basement membrane discovered during examination under the electron-microscope has been established. Ultimately, the disease has been described as a condition characterized with a diverse clinical course, usually mild, but sometimes progressive. It is a family conditioned disease, but it also appears sporadically and concerns at least 1% of the population. It has also been stated that it is one of the most frequent renal diseases, enumerated directly after changes caused by infections, hypertension and renal lithiasis. This particular disease is diagnosed more often than IgA nephropathy and Alport syndrome, which are also associated with haematuria or microhematuria.

Keywords: thin basement membrane disease.

Introduction

Initially, the thin glomerular basement membrane disease was called "a gentle and curable hemorrhagic nephritis". G. Baehr presented this name in 1926 [1]. The author described this disease in 14 young adults, who temporarily suffered from painless microhematuria. The course of this condition did not reveal hypertension or oedemas. The author concluded that prognosis concerning such cases are clearly optimistic. In 1966 McConville and McAdams reported occurrence of mild haematuria, which was conditioned not only by family factors, but also by factors not related with family. In case of occurrence associated with genetic conditions, they proved that this condition reveals an autosomal dominant inheritance pattern [2].

The thin basement membrane disease has been finally characterized at the beginning of 1970s [3]. This is when the connection between previously clinically described gentle microhematuria and significant thinning of glomerular basement membrane discovered during examination under the electron-microscope has been established. Some authors initially believed that this disease does not stand as the final reason underlying renal insufficiency. What is more, medical inquiries did not reveal any information on the occurrence of uraemia among patients' family members [4]. There were descriptions of cases, where haematuria was present along with proteinuria. Some patients suffered from renal insufficiency [5].

Ultimately, the disease has been described as a condition characterized with a diverse clinical course, usually mild, but sometimes progressive. It is a family conditioned disease, but it also appears sporadically and concerns at least 1% of the population [6]. It has also been stated that it is one of the most frequent renal diseases, enumerated directly after changes caused by infections, hypertension and renal lithiasis [7]. This particular disease is diagnosed more often than IgA nephropathy and Alport syndrome, which are also associated with haematuria or microhematuria [8].

Clinical symptoms

In children, this condition is usually recognized at the age of about 7, whereas in adults, it is diagnosed at

the age of about 37. There are, however, many cases in which the disease appears at a later age. Some authors declare the lack of differences in the frequency of occurrence depending on the gender [8]. Others state that women tend to dominate among patients suffering from this disease [9]. In more than 30% of patients no family history of the disease can be stated [8]. Microhematuria is the most often observed symptom, sometimes episodes of haematuria are also reported. It usually occurs after physical exercises, and also as a result of infection. Slight or moderate proteinuria can also appear during the course of the disease. Nephritic syndrome is very rare in this case.

Histological image

Histological image does not reveal any characteristic features. Glomerular changes are usually imperceptible [10]. Presence of erythrocytes in the tubule lumen is a quite frequently described lesion [11]. It is impossible to present thinning of basement membranes under optical microscope. However, in some cases it is possible to observe their decreased silver absorption [8, 12–15]. What can also be observed is the increased number of mesangial cells with matrix expansion.

Biopsies performed in children rarely do reveal chronic lesions. Whereas, as far as adults are concerned, it is possible to diagnose various degrees of sclerosing glomerular lesions, as well as focuses of interstitial fibrosis and changes in interstitial blood vessels [16]. These changes are most often associated with hypertension or senile age of patients. Nevertheless, in some patients the focal segmental glomerulosclerosis appears prior to the occurrence of hypertension and proteinuria. It has been assumed that such premature glomerulosclerosis may indicate the risk of disease progression [17].

In majority of patients, immunofluorescent examinations do not reveal presence of concrements [17, 18, 19, 20]. Sometimes small, single IgG, IgM, IgG or C3 concrements can be visible [17, 20].

Electron-microscopic image

Thin basement membrane disease is diagnosed when examination under the electron-microscope reveals thinning of lamina densa in the basement membrane, which also extensively covers the glomerulus. The diagnosis seems apparently easy, but we can face certain problems at this stage [14]. One of them is the fact that there are no common criteria for evaluating proper thickness of the basement membrane. Some authors [13] have assumed that the thickness of glomerular basement membrane in an infant equals 150 nm and in one-year-old child it reaches 200 nm. Whilst in children between the 1st and the 6th year of age it ranges between 208 and 245 nm, and between the age of 6 and 11 it is only slightly thicker, reaching 244–307 nm. In adult women this value reaches 320 ± 50 nm, whereas in adult men it can reach 370 ± 50 nm.

It has been assumed that the lower margin of proper basement membrane among adults should not be less than 200–265 nm. Steffes et al. [21] assessed that up to the age of three the basement membrane has not more than 200 nm, in adult females it has 323 ± 45 nm, and in adult males it reaches 373 ± 42 nm. According to guidelines elaborated by the World Heath Organisation helping to diagnose the thin basement membrane disease, it is essential to assume the 250 nm threshold for adults and the 180 nm threshold for children between 2 and 11 years of age [22]. A certain group of authors report that the membrane thickness in the course of this disease ranges between 100 and 250 nm. Other authors believe that this disease can be diagnosed when membrane thickness does not exceed 200 nm [23]. It has been shown that the thickness of membranes increases during the first 2 years of life of a healthy child and reaches 200–300 nm [24, 25]. There are authors [26] who confirmed the data above, but they also simultaneously observed that the lamina densa itself takes much longer to form, as it is not fully formed until the age of four. In the light of this information, all statements calling for exclusion of morphological tests in children under the age of 10 seem entirely groundless [18].

In order to evaluate the thickness of basement membranes, laboratories tend to use various measuring methods. Most authors take advantage of data derived from multiple measures, which are performed on peripheral parts of the loop of capillary vessels. Usually the measurement of basement membrane is taken from basement cell membrane of part in podocytes to the cell membrane in the endothelium. During such measurement the thickness of the whole basement membrane is assessed. Some authors recommend limiting measures only to the thickness of lamina densa. Decreased thickness of lamina densa is of crucial significance in diagnosing thin basement membrane disease [4, 27]. There is no definite opinion on the topic of the assessment of the extent of these changes. It is assumed that the diagnosis of thin basement membrane disease depends on the discovery of extensive thinning involving the globeruli in electron-microscopic examinations. It has not been clearly defined, however,

how the term „extensive" is to be understood. Sue et al. [28] define thin basement membrane disease as a uniform thinning including at least 50% of the membranes. Monnens [29], who confirms that the thinning of the membranes is extensive, says that the thinning can sometimes be focal, but must concern at least 50% of the membranes present in the submitted material. Savige et al. [7] assume that the thinning of the membranes must concern the majority of the capillaries and in each capillary 50% of the length of the membranes.

Apart from significant thinning of membranes, sometimes a change in its structures can also be observed, or even their discontinuation. These changes facilitate migration of red blood cells into the ultrafiltrate [30, 31].

Genetic origin of the thin basement membrane disease

Lemmink et al. [32] were the first ones to describe the mutation in COL4A3/COL4A4 genes in patients with mild forms of microhematuria, and then described mutation in locus COL4A4, which is responsible for changes in glycine substitution in the place of glutamic acid within the collagen area of the gene. They suggested that patients with family mild microhematuria could be heterozygous carriers of the autosomal recessive Alport syndrome (ARAS) [32]. These particular mutations concern the same places that are responsible for autosomal inheritance of the Alport syndrome. They are point sites, and they concern the coding region. Until now no mutation within the region of COL4A3/COL4A4 gene promoter in thin basement membrane disease has been described. Nonetheless, such mutations have been documented in case of the Alport syndrome [13].

Apart from the research on the genetic differences between Alport syndrome and thin basement membrane disease, the subject literature gives only minor attention to the differentiation of these two diseases. In morphological research, though, Alport syndrome itself and the diagnostic difficulties, especially in the early development stage, are often emphasised. The depletions of the structure of the lamina densa typical for Alport syndrome occur also in thin basement membrane disease, but Alport syndrome is sometimes characterized by a lasting thinning of the lamina densa without the characteristic stratification with granulocytic inclusions between the layers [33].

Thin membranes in different glomerulopathies

The presence of thin basement membranes is observed in different glomerulopathies, such as IgA nephropathy, membranoproliferative glomerulonephritis, membranous glomerulonephritis, lupus nephritis, acute endocapillary proliferative glomerulonephritis, extracapillary glomerulonephritis, diabetic nephropathies, tubulointerstitial nephritis, Fabry disease and FSGS [34–43].

Taking the data presented above into consideration, diagnosis concerning thin basement membrane disease cannot be described as an easy procedure. This disease is not usually considered during clinical consideration and not widely accounted for in morphological diagnostics.

Acknowledgements

KBN nr N N402 482037

Conflict of interest statement
The authors declare that there is no conflict of interest in the authorship or publication of contribution.

Funding sources
There are no sources of funding to declare.

References

1. Baehr G. Benign and curable form of hemorrhagic nephritis. Jama. 1926;86:1001–1004.
2. McConville JM, McAdams AJ. Familial and nonfamilial benin hematuria. J Pediatr. 1966;2:207–214.
3. Rogers PW, Kurtzman NA, Bunn SM Jr, White MG. Familial benign essential hematuria. Arch Intern Med. 1973; 131:257–262.
4. Kashtan C. Alport syndrome and thin basement membrane nephropathy: diseases arising from mutation in type IV collage. Saudi J Kidney Dis Transplant. 2003;14:276–289.
5. Dische FE, Weston MJ, Parsons V. Abnormally thin glomerular basement membran es associated with hematuria, proteinuria or renal failure in adults. Am J Nephrol. 1985;5:103–109.
6. Wang YY, Savigne J. The epidemiology of thin basement membrane nephropathy. Semin Nephrol. 2005;25:136–139.
7. Savige J, Rana K, Tonna S, Buzza M, Dagner H, Yan Yan Wang: Thin basement membrane nephropathy Kidney Int. 2003;64:1169–1178.
8. Hennigar RA, Tumlin JA: Glomerular diseases associated primarily with asymptomatic or gross hematuria in Silva's Diagnostic Renal Pathology ed. By Xin J. Zhou and col. Cambridge University Press: 177.
9. Colvin RB: Thin basement membrane disease in Diagnostic Pathology, Kidney Disease. Amirsys. 2011: 296–297.
10. Tina L, Jenis E, Jose P, Medani C, Papadopoulou Z, Calcagno P. The glomerular basement membrane in Benin familial hematuria. Clin Nephrol. 1982;17:1–4.
11. Waldherr R. Familial glomerular disease. Contrib Nephrol. 1982;33:104–121.
12. Kriz W. Ontogenetic development of the filtration barrier. Nephron Exp Nephrol. 2007;106:44–50.

13. Tryggvason K, Patrakka J. Thin Basement Membrane Nephropathy J Am Soc Nephrol. 2006;17:813–822.
14. Foster K, Markowitz GS, D'Agati VD: Pathology of thin basement membrane nephropathy. Semin Nephrol. 2005;25:149–158.
15. van Breda Vriesman PJC. Thin glomerular basement membrane nephropathy in adults. Nephron. 1998;79:1–7.
16. Nieuwhof CMG, de Heer F, de Leeuw P, van Breda Vriesman PJC. Thin GBM nrphropathy: premature obsolence is associated with hypertension and late onset renal failure. Kidney Int. 1997;51:1596–1601.
17. Yoshikawa N, Hashimoto H, Katayama Y, Yamada Y, Matsuo T. The thin glomerular basement membrane in children with hematuria. J Pathol. 1984;142:253–257.
18. Gautier B, Trachtman H, Frank R, Valderrama E. Familial thin basement nephropathy in children with asymptomatic microhematuria. Nephron. 1989;51:502–508.
19. Gautier B, Trachtman H. Asymptomatic hematuria. Pediatr Nephrol. 1990;4:296–302.
20. Piel CF, Biava CG, Goodman JR: Glomerular basement membrane attenuation in famialial nephritis and "benign" hematuria. J Pediatr. 1982;101:358–365.
21. Steffes MW, Barbosa J, Basgen JM, Sutherland DER, Najarian JS, Maner SM: Quantitative glomerular morphology of the normal kidney. Lab Invest. 1983;49:82–86.
22. Churg J, Bernstein J, Glassock R. Renal Disease: Classification and Atlas of Glomerular Diseases, 2nd ed., Tokyo, Japan: Igaku-Shoin. 1995.
23. Mandache E, Gherghiceanu M. Ultrastructural defects of the glomerular basement membrane associated with primary glomerular nephropathies. Ultrastruct Pathol. 2004;28:103–108.
24. Trachtman H, Weiss RA, Benett B, Greifer J. Isolated hematuria in children; indication for a renal biopsy. Kidney Int. 1984;25:94–99.
25. Rumpelt HJ: Alport`s syndrome: specifity and pathogenesis of glomerular basement membrane alterations. Pediatr Nephrol. 1987;1:422–427.
26. Vogler C, McAdams J, Homan Sharon M. Glomerular basement membrane and lamina densa n infants and children. Pediatr Pathology. 1987;7:527–534.
27. Meleg-Smith S. Alport disease: a review of the diagnostic difficulties. Ultrastruct Pathol. 2001;25:193–200.
28. Sue YM, Huang JJ, Hsieh RY, Chen FF: Clinical features of thin basement membrane disease and associated glomerulopathies. Nephrol. 2004;9:14–18.
29. Monnens LAH. Thin glomerular basement membrane disease. Kidney Int. 2001;60:799–800.
30. Collar JE, Ladva S, Cains TDA, Cattel V. Red cell transverse througt thin glomerular basement mambranes. Kidney Int. 2001;59:2069–2072.
31. Lapis H, Foster K, Miner JH: Red cell transverse through thin glomerular basement membrane. Kidney Int. 2002;61:762–763.
32. Lemmink HH, Nillesen WN, Mochizuki T, Schröder CH, Brunner HG, van Oost BA, Monnens LA, Smeets HJ: Benign familial hematuria due to mutation of the type IV collagen alpha4 gene. J Clin Invest. 1996;98:1114–1118.
33. Liapis H, Gokden N, Hmiel P, Miner JH: Histopathology, ultrastructure, and clinical phenotypes in thin glomerular basement membrane disease variants. Hum Pathol. 2002;33:836–845.
34. Jayakumar VR, Hinduja A, Georgi A, Soundarajan P, Kuruvilla S, Veukateshan S. IgA nephropathy and FSGS in twoo patients with thin glomerular membrane. Indian J Nephrol. 2001;11:27–29.
35. Berhoux FC, Laurent B, Alamartine E, Diab E. _ew subgroup of primary IgA nephritis with thin basement membrane (GBM): syndrome or association. Nephrol Dial Transplant. 1996;11:558–561.
36. Danilewicz M, Wągrowska-Danilewicz M. Glomerular basement membrane thickness in primary diffuse IgA nephropathy: ultrastructural morphometric analysis. Int Urol Nephrol. 1998;30:513–519.
37. Allwardt R, Savige J, Wilson D. A. Comparison of the clinical and laboratory features of thin basement membrane disease and IgA glomerulonephritis. Clin Nephrol. 1999;52:1–4.
38. Fujiki Y, Nagase M, Kobayashi S, Honda N, Muranuka Y. Alteration of glomerular basement membrane relevant to hematuria. Virchows Arch A Pathol Anat. 1988;43:159–165.
39. Frasca GM, Soverini L, Gharavi AG, Lifton RP, Canova C, Preda P, Vangalista A, Stefoni S. Thin basement membrane desease in patients with familial IgA nephropathy. J Nephrol. 2004;17:778–785.
40. Lanteri M, Wilson D, Savige J. Clinical features in two patients with IgA glomerulonephritis and thin – basement membrane disease. Nephrol Dial Transplant. 1996;11:791–793.
41. Monga G, Mazzucco G, Roccatello D. The association of IgA glomerulonephritis and thin basement membrane disease in a hematuric patient: light, electron microscopic and immunofluorescence investigation. Am J Kidney Dis. 1991;18:409–412.
42. Ueno M. Thin basement membrane disease in patients with asymptomatic hematuria and/or proteinuria: A clinicopathological study. Nippon Jinzo Gakkai Shi. 1991; 33:339–347.
43. Yoshida K, Suzuki J, Suzuki S, Kume K, Suzuki H, Hujiki T. A case of IgA nephropathy in tree sisters with thin basement membrane disease. Am J Nephrol. 1998;18:422–424.

Acceptance for editing: 2015-02-10
Acceptance for publication: 2015-03-30

Correspondence address:
Jakub Żurawski
8 Rokietnicka Str., 60-806 Poznańn, Poland
phone: +48 61 8547652
email: zibc@ump.edu.pl

Journal of Medical Science
Instructions for Authors

Journal of Medical Science (JMS) is a PEER-REVIEWED, OPEN ACCESS journal that publishes original research articles and reviews which cover all aspects of clinical and basic science research. The journal particularly encourages submissions on the latest achievements of world medicine and related disciplines. JMS is published quarterly by Poznan University of Medical Sciences.

ONLINE SUBMISSION:
Manuscripts should be submitted to the Editorial Office by an e-mail attachment: nowinylekarskie@ump.edu.pl. You do not need to mail any paper copies of your manuscript.

All submissions should be prepared with the following files:
- Cover Letter
- Manuscript
- Tables
- Figures
- Supplementary Online Material

COVER LETTER: *Manuscripts* must be accompanied by a *cover letter* from the author who will be responsible for correspondence regarding the manuscript as well as for communications among authors regarding revisions and approval of proofs. The cover letter should contain the following elements: (1) the full title of the manuscript, (2) the category of the manuscript being submitted (e.g. Original Article, Brief Report), (3) the statement that the manuscript has not been published and is not under consideration for publication in any other journal, (4) the statement that all authors approved the manuscript and its submission to the journal, and (5) a list of at least two referees.

MANUSCRIPT: Journal of Medical Science publishes Original Articles, Brief Reports, Review articles, Mini-Reviews, Images in Clinical Medicine and The Rationale and Design and Methods of New Studies. From 2014, only articles in English will be considered for publication. They should be organized as follows: Title page, Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgments, Conflict of Interest, References and Figure Legends. All manuscripts should be typed in Arial or Times New Roman font and double spaced with a 2,5 cm (1 inch) margin on all sides. They should be saved in DOC, DOCX, ODT, RTF or TXT format. Pages should be numbered consecutively, beginning with the title page.

Ethical Guidelines
Authors should follow the principles outlined in the Declaration of Helsinki of the World Medical Association (www.wma.net). The manuscript should contain a statement that the work has been approved by the relevant institutional review boards or ethics committees and that all human participants gave informed consent to the work. This statement should appear in the Material and Methods section. Identifying information, including patients' names, initials, or hospital numbers, should not be published in written descriptions, illustrations, and pedigrees. Studies involving experiments with animals must be conducted with approval by the local animal care committee and state that their care was in accordance with institution and international guidelines.

Authorship:
According to the International Committee on Medical Journal Ethics (ICMJE), an author is defined as one who has made substantial contributions to the conception and development of a manuscript. Authorship should be based on all of the following: 1) substantial contributions to conception and design, data analysis and interpretation; 2) article drafting or critical advice for important intellectual content; and 3) final approval of the version to be published. All other contributors should be listed as acknowledgments. All submissions are expected to comply with the above definition.

Conflict of Interest
The manuscript should contain a conflict of interest statement from each author. Authors should disclose all financial and personal relationships that could influence their work or declare the absence of any conflict of interest. Author's conflict of interest should be included under Acknowledgements section.

Abbreviations
Abbreviations should be defined at first mention, by putting abbreviation between brackets after the full text. Ensure consistency of abbreviations throughout the article. Avoid using them in the title and abstract. Abbreviations may be used in tables and figures if they are defined in the table footnotes and figure legends.

Trade names
For products used in experiments or methods (particularly those referred to by a trade name), give the manufacturer's full name and location (in parentheses). When possible, use generic names of drugs.

Title page
The first page of the manuscript should contain the title of the article, authors' full names without degrees or titles, authors' institutional affiliations including city and country and a running title, not exceeding 40 letters and spaces. The first page should also include the full postal address, e-mail address, and telephone and fax numbers of the corresponding author.

Abstract
The abstract should not exceed 250 words and should be structured into separate sections: Background, Methods, Results and Conclusions. It should concisely state the significant findings without reference to the rest of the paper. The abstract should be followed by a list of 3 to 6 Key words. They should reflect the central topic of the article (avoid words already used in the title).

The following categories of articles can be proposed to the Journal of Medical Science:

ORIGINAL RESEARCH

Original articles: Manuscripts in this category describe the results of original research conducted in the broad area of life science and medicine. The manuscript should be presented in the format of Abstract (250-word limit), Keywords, Introduction, Material and Methods, Results, Discussion, Perspectives, Acknowledgments and References. In the Discussion section, statements regarding the importance and *novelty of the study* should be presented. In addition, the limitations of the study should be articulated. The abstract must be structured and include: Objectives, Material and Methods, Results and Conclusions. Manuscripts cannot exceed 3500 words in length (excluding title page, abstract and references) and contain no more than a combination of 8 tables and/or figures. The number of references should not exceed 45.

Brief Reports: Manuscripts in this category may present results of studies involving small sample sizes, introduce new methodologies, describe preliminary findings or replication studies. The manuscript must follow the same format requirements as full length manuscripts. Brief reports should be up to 2000 words (excluding title page, abstract and references) and can include up to 3 tables and/or figures. The number of references should not exceed 25.

REVIEW ARTICLES

Review articles: These articles should describe recent advances in areas within the Journal's scope. Review articles cannot exceed 5000 words length (excluding title page, abstract and references) and contain no more than a combination of 10 tables and/or figures. Authors are encouraged to restrict figures and tables to essential data that cannot be described in the text. The number of references should not exceed 80.

A THOUSAND WORDS ABOUT... is a form of Mini-Reviews. Manuscripts in this category should focus on *latest achievements of life science and medicine.* Manuscripts should be up to 1000 words in length (excluding title page, abstract and references) and contain up to 5 tables and/or figures and up to 25 most relevant references. The number of authors is limited to no more than 3.

OTHER SUBMISSIONS

Invited Editorials: Editorials are authoritative commentaries on topics of current interest or that relate to articles published in the same issue. Manuscripts should be up to 1500 words in length. The number of references should not exceed 10. The number of authors is limited to no more than 2.

Images in Clinical Medicine: Manuscripts in this category should contain one distinct image from life science or medicine. Only original and high-quality images are considered for publication. The description of the image (up to 250 words) should present relevant information like short description of the patient's history, clinical findings and course, imaging techniques or molecular biology techniques (e.g. blotting techniques or immunostaining). All labeled structures in the image should be described and explained in the legend. The number of references should not exceed 5. The number of authors is limited to no more than 5.

The Rationale, Design and Methods of New Studies: Manuscripts in this category should provide information regarding the grants awarded by different founding agencies, e.g. National Health Institute, European Union, National Science Center or National Center for Research and Development. The manuscript should be presented in the format of Research Project Objectives, Research Plan and Basic Concept, Research Methodology, Measurable Effects and Expected Results. The article should also contain general information about the grant: grant title, keywords (up to five), name of the principal investigator and co-investigators, founding source with the grant number, *Ethical Committee permission number,* code in clinical trials (if applicable). Only grant projects in the amount over 100,000 Euro can be presented. Manuscripts should be up to 2000 words in length (excluding references) and can include up to 5 tables and/or figures. The abstract should not exceed 150 words. The number of authors is limited to the Principal Investigator and Co-investigators.

Acknowledgements
Under acknowledgements please specify contributors to the article other than the authors accredited. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.). Also acknowledge all sources of support (grants from government agencies, private foundations, etc.). The names of funding organizations should be written in full.

References
All manuscripts should use the 'Vancouver' style for references. References should be numbered consecutively in the order in which they appear in the text **and listed at the end of the paper.** References cited only in Figures/Tables should be listed in the end. Reference citations in the text should be identified by Arabic numbers in square brackets. Some examples:

This result was later contradicted by Smith and Murray [3].
Smith [8] has argued that...
Multiple clinical trials [4–6, 9] show...

List all authors if there are six or fewer; if there are seven or more, list first six follower by "et al.". Journal names should be abbreviated according to Index Medicus.

Some examples
Standard journal articles

1. Fassone E, Rahman S. Complex I deficiency: clinical features, biochemistry and molecular genetics. J Med Genet. 2012 Sep;49(9):578–590.

2. Pugh TJ, Morozova O, Attiyeh EF, Asgharzadeh S, Wei JS, Auclair D et al. The genetic landscape of high-risk neuroblastoma. Nat Genet. 2013 Mar;45(3):279–284.

Books
Personal author(s)

1. Rang HP, Dale MM, Ritter JM, Moore PK. Pharmacology. 5th ed. Edinburgh: Churchill Livingstone; 2003.

Editor(s) or compiler(s) as authors

2. Beers MH, Porter RS, Jones TV, Kaplan JL, Berkwits M (editors). The Merck manual of diagnosis and therapy. 18th ed. Whitehouse Station (NJ): Merck Research Laboratories; 2006.

Chapter in the book

1. Phillips SJ, Whisnant JP. Hypertension and stroke. In: Laragh JH, Brenner BM, editors. Hypertension: pathophysiology, diagnosis, and management. 2nd ed. New York: Raven Press; 1995. p. 465–478.

TABLES: Tables should be typed on sheets separate from the text (each table on a separate sheet). They should be numbered consecutively with Arabic numerals. Tables should always be cited in text (e.g. table 2) in consecutive numerical order. Each table should include a compulsory, concise explanatory title and an explanatory legend. Footnotes to tables should be typed below the table body and referred to by superscript lowercase letters. No vertical rules should be used. Tables should not duplicate results presented elsewhere in the manuscript (e.g. in figures).

FIGURES: All illustrations, graphs, drawings, or photographs are referred to as figures and must be uploaded as separate files when submitting a manuscript. Figures should be numbered in sequence with Arabic numerals. They should always be cited in text (e.g. figure 3) in consecutive numerical order. Figures for publication must only be submitted in high-resolution TIFF or EPS format (*minimum 300 dpi resolution).* Each figure should be self-explanatory without reference to the text and have a concise but descriptive legend. All symbols and abbreviations used in the figure must be defined, unless they are common abbreviations or have already been defined in the text. Figure Legends must be included after the reference section of the Main Text.

Color figures: Figures and photographs will be reproduced in full colour in the online edition of the journal. In the paper edition, all figures and photographs will be reproduced as black-and-white.

SUPPLEMENTARY ONLINE MATERIAL: Authors may submit supplementary material for their articles to be posted in the electronic version of the journal. To be accepted for posting, supplementary materials must be essential to the scientific integrity and excellence of the paper. The supplementary material is subject to the same editorial standards and peer-review procedures as the print publication.

Review Process
All manuscripts are reviewed by the Editor-in-Chief or one of the members of the Editorial Board, who may decide to reject the paper or send it for external peer review. Manuscripts accepted for peer review will be blind reviewed by at least two experts in the field. After peer review, the Editor-in-Chief will study the paper together with reviewer comments to make one of the following decisions: accept, accept pending minor revision, accept pending major revision, or reject. Authors will receive comments on the manuscript regardless of the decision. In the event that a manuscript is accepted pending revision, the author will be responsible for completing the revision within 60 days.

Copyright