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Adequacy of Trephine Bone Marrow Biopsies: The Doctor and the Patient Make a Difference*

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Reports of 1938 trephines submitted from five institutions over a 30-month period were analyzed looking for associations between the hospital of origin, operator, bone marrow pathology, patient's age and the biopsy quality. The arbitrary adequacy criteria (min 10 mm of interpretable marrow or min 10 intertrabecular spaces) were fulfilled by 61.9% of the biopsies. The performance of individual operators varied from 15.9% to 87.8% of adequate trephines. The group of doctors performing more than 100 biopsies in the study period had satisfactory results. The intermediate group (20–100 biopsies) was the least homogenous, and on the average had the poorest biopsy quality. The biopsy quality was influenced by diagnostic categories, correlated positively with bone marrow fibrosis and negatively with the patient's age. The trephine quality in practice may be lower than the published or declared standards. Ideally the procedure should be executed by the practitioners making more than one trephine a week. Prior to the biopsy it is possible to estimate the level of difficulty posed by an individual patient and use this information to minimize the risk of obtaining an inadequate core.

Introduction

Trephine bone marrow biopsy belongs to one of the most important diagnostic tools in hematology, offering an insight into the histology of hematopoietic tissue, including its stroma and the bone framework. Originally limited to the instances of previous unsuccessful aspiration ("dry tap"), it gained popularity in the last decades, and now its indications almost overlap with these for an aspiration (cytological) biopsy [1]. The major strength of a trephine biopsy is in visualization of topography of the bone marrow, particularly abnormal clustering or abnormal location of cells, assessment of the bone marrow stroma (edema, fibrosis, etc), and visualization of bone trabecule. A good trephine (Fig. 1) shows nearly two logs more cells than it is assessed using a routine set of smears[1], which is particularly important in the analysis of relatively rare events, like megakaryocytes, specific types of macrophages (for instance pseudo-Gaucher cells) or non-hematopoietic metastases. However, the details of morphology of individual cells are much better seen in the smears than in the trephines, so in practice both methods are highly complementary. The trephine bone marrow biopsies are traditionally performed by clinicians, usually hematologists, and assessed microscopically by specially trained pathologists.

The trephine bone marrow biopsy procedure is relatively safe [2], but usually uncomfortable for the patients, so particular care should be assigned to obtain high-quality cores, paying off the patient's suffering with meaningful diagnostic data. In an everyday practice we encounter a very big diversity in the quality of the trephines, and different reasons behind its frequent technical inadequacy. We undertook this study to shed some light onto the possible factors influencing the adequacy of the trephine bone marrow biopsies. Designing this audit we had a subjective impression that the quality of the biopsies may depend not only on the skills of an operator, but also on some factors related to the patient, particularly to the specific bone marrow pathology. This study was also aimed at providing some practical guidelines for practitioners performing this procedure.

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[1]A standard high power field (lens 40×, area of 0.24 mm^2), with 100% cellularity contains approximately 2250 hematopoietic cells. This makes approximately 5,000 cells per square millimeter in a bone marrow with 50% cellularity and over 50,000 cells per one whole cross-section through an adequate trephine biopsy. Usually at least 5 cross-sections at different levels are analyzed.

Material and Methods

The audit was based on trephine bone marrow biopsies collected in the Department of Pathomorphology, Collegium Medicum, Jagiellonian University from 1 May 2001 to 31 October 2003, and submitted from the institutions sending more than 40 biopsies in the study period. Several institutions submitting smaller numbers of biopsies were excluded. Those were either the hospitals only exceptionally performing this procedure (obtained small groups would be impractical in the subsequent statistical analysis), or the hospitals generally submitting their trephines elsewhere (the consultation cases we received could be biased in terms of their quality). In all the hospitals taken into account the trephines were obtained using the adult-type Jamshidi needle, under local anesthesia. The routine processing included acid-based decalcification using commercially available medium (D-decalcifier, Shandon, Pittsburgh, PA), paraffin embedding and cutting into the 3–4 micron sections, stained with hematoxylin/eosin, Giemsa, periodic-acid Schiff and Gomori silver, and other special stains when appropriate. The trephine length measurement was performed upon processing, on histological slides, and was limited to the total area occupied by interpretable bone marrow (i.e. the crushed marrow, cortical bone, cartilage, periosteum and more superficial tissues were ignored).

The trephine bone marrow biopsy was reported adequate (representative) if it contained at least 10 mm of technically satisfactory bone marrow or at least 10 fully preserved intertrabecular spaces. The tangential biopsies containing exclusively the fat tissue were considered inadequate due to a well-known phenomenon of local subcortical aplasia constituting a variant of the histological norm [3]. The areas identifiable as a previous biopsy site were not considered as belonging to the adequate part of the biopsy.

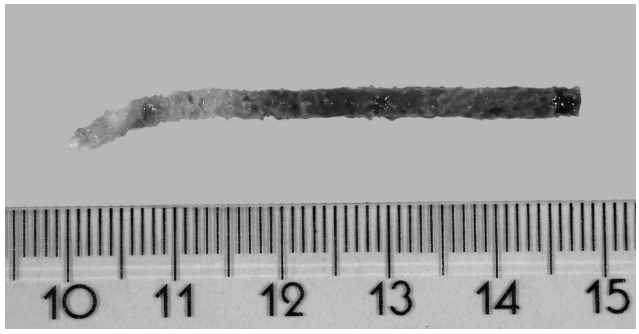

Fig. 1. Gross photography of a representative trephine bone marrow biopsy core after a brief routine formaldehyde fixation. Intertrabecular spaces and "honeycomb"-like trabecular bone marrow scaffolding are clearly visible without any magnifying devices. On the right the cortical bone can be seen.

The "operator factors" possibly influencing the biopsy quality included the hospital, the time of the procedure, and the name of a doctor identified as performing the biopsy from the requisition forms. The "patient factors" encompassed the patient's age, sex, disease, degree of bone marrow fibrosis and osteopenia. For the sake of a meaningful statistical analysis we arbitrarily grouped the diagnoses into 9 working categories: acute lymphoblastic leukemias (ALL), acute myeloid leukemias (AML), chronic lymphocytic leukemia (CLL), lymphomas other than CLL, myelodysplastic syndromes (MDS), multiple myeloma (MM), chronic myeloproliferative disorders (CMPD), nonspecific changes and others.

A case was assigned to one of the neoplastic categories (ALL, AML, CLL, lymphoma, MDS, MM or CMPD) only when the bone marrow in the trephine biopsy contained any identifiable neoplastic infiltrate, even the minimal residual disease. The "nonspecific" group was designed to investigate the cases with a more or less normal bone marrow, so the negative post-treatment biopsies or the negative staging trephines from the patients with a prior diagnosis of one of these neoplasms mostly fell into the "nonspecific" category. The CLL group was limited to the B-cell tumors, but encompassed also the small lymphocytic lymphoma (SLL). The "lymphoma" category excluded CLL/SLL, but included the Hodgkin disease. The MDS/MPD overlap syndromes were lumped with the MDS cases. The "others" group was created to consider the rare and diverse conditions, largely to be eliminated from the further analysis due to a small number of cases. Thus it contained rare infiltrative diseases of bone marrow (disseminated non-hematopoietic tumors, Gaucher disease), bone marrow aplasia or intense degeneration (total or subtotal necrosis or gelatinous transformation), and finally these trephines in which extreme technical inadequacy completely precluded any, even tentative, diagnosis.

The bone marrow stromal fibrosis was originally recorded in a five-grade semi-quantitative scale [4], but for the subsequent statistics it was reduced to 3 categories: normal (0/+1), mild fibrosis (+2), strong fibrosis (+3/+4). Osteopenia was analyzed as a yes/no phenomenon, upon the diagnosis based on the standard guidelines [3].

The statistical analysis encompassed the time-series methods, logistic regression, and Mann-Whithey U or χ^2 tests, when appropriate. The significance level was set at 0.05. All computations were performed with Statistica 6.0 PL software (StatSoft, Inc., USA).

A standardized questionnaire was sent to the members of European Bone Marrow Working Group (EBMWG) to obtain the information on their working criteria for an adequate trephine bone marrow biopsy.

Results

The "Doctor" factors

Of the total 1938 trephine bone marrow biopsies in the study period 1201 (61.97%) were considered adequate by our arbitrary criteria.

The length of the interpretable bone marrow part of the trephines varied between 0 to 57 mm (mean 13.1 ± 8.3 mm, median 11 mm). The Figure 2 shows the skewed distribution of bone marrow length of the cores. The median number of intertrabecular spaces was 10, maximum 131.

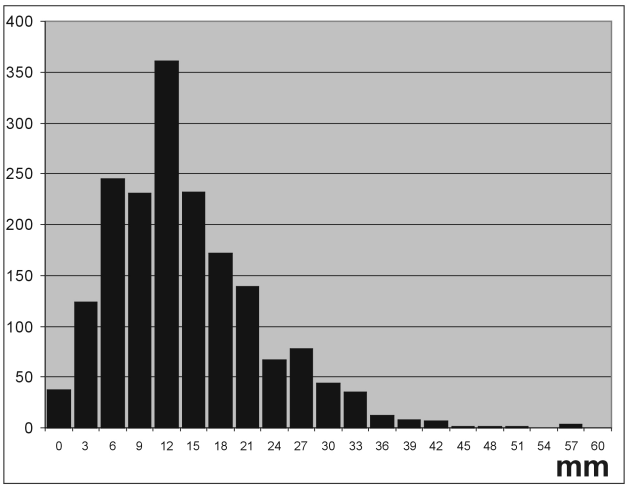

Fig. 2. Length distribution of 1938 trephine biopsies (interpretable bone marrow only) submitted from five hospitals to the Department of Pathomorphology, Jagiellonian University during 30 consecutive months.

Of the 737 inadequate trephines 662 (90%) were too small to fulfill our minimal criteria, 181 (24.6%) were crushed, 13 (1.8%) extensively permeated with blood or serum. Additionally 31 (4.2%) biopsies were tangential and inadequate due to visualization of subcortical fat and 15 (2%) contained predominantly the site of the previous biopsy. These reasons behind the inadequacy to some extent overlapped, for instance of 181 crushed trephines only 26 (14.4%) were large enough to be considered adequate if not mechanically damaged.

Five hospitals submitted more than 40 biopsies and were considered in the analysis of possible differences in the trephine quality between referring centers. The number of biopsies submitted in the study period varied from 44 (less than 2 biopsies per month) to 1683 (56 biopsies per month). The percentage of adequate biopsies ranged from 15.9% to 77.4%, and differed significantly between the hospitals (χ^2 $p<0.00001$, Table 1). There was no correlation between the number of biopsies and their quality at the hospital level.

To test for the performance of the individual operators we analyzed the quality of biopsies for 31 clinicians submitting more than 10 trephines in the study period. The individual success rate varied from 15.9% to 87.8% of adequate trephines (Fig. 3). Three groups of doctors – these performing a large number of biopsies (over 100 in the study period), performing 100 – 20 biopsies, and performing below 20 biopsies – showed differences in the percentage of adequate trephines (Fig. 3). The most experienced operators had a relatively high success rate with low inter-individual variability (66.9% ± 7.2% of adequate trephines) and differed significantly from all other doctors taken together (55.7% ± 15.6, χ^2 $p=0.0000012$), and from the very inhomogeneous median group (54.0% ± 18.5%, χ^2 $p=0.0000002$, $F=0.049$). Unexpectedly, there was a marginal tendency towards better performance of the infrequent operators (below 20 biopsies, 62.3% ± 8.3%) over the medium group (χ^2 $p=0.056$). There were no statistical differences between the groups of frequent (>100 trephines) and infrequent (<20 trephines) operators.

The quality of biopsies varied also in the study period, showing not a steady trend, but rather a seasonal variation demonstrating some periodicity (Fig. 4). The periods of the poorest performance were corresponding to each of the three summers within the study time span. Additionally

TABLE 1

Quality of trephine bone marrow biopsies submitted from 5 hospitals over the 30-month study period (May 2001–October 2003). The differences in percentage of adequate cores are significant with χ^2 $p<0.00001$.

Hospital	Number of biopsies in the 30-month period	% of adequate biopsies	Trephine length (mm) median (inter-quartile range)	Number of intertrabecular spaces median (inter-quartile range)
A	44	15.9%	5 (4 – 10)	4 (2 – 5.5)
B	53	77.4%	15 (10 – 19)	12 (9 – 16)
C	47	42.6%	8 (5 – 13)	7 (4 – 10)
D	111	51.3%	10 (7 – 12)	10 (7 – 14)
E	1683	63.9%	12 (7 – 18)	10 (6 – 17)

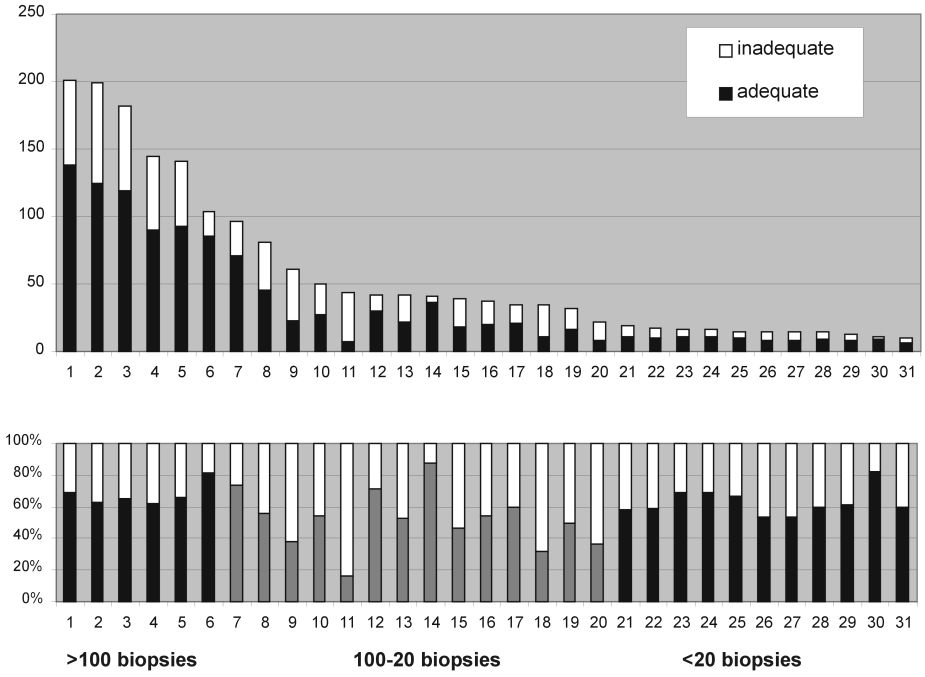

Fig. 3. Absolute numbers (above) and percentages (below) of adequate and inadequate trephines produced by each operator who performed more than 10 trephines in the study period. The operators are ranked by the number of biopsies. The medium group (*20-100 biopsies*, gray color on the lower panel) produced statistically poorer biopsies than the *>100 biopsies* group and the *<20 biopsies* group, and showed the greatest intra-group diversification in the biopsy quality.

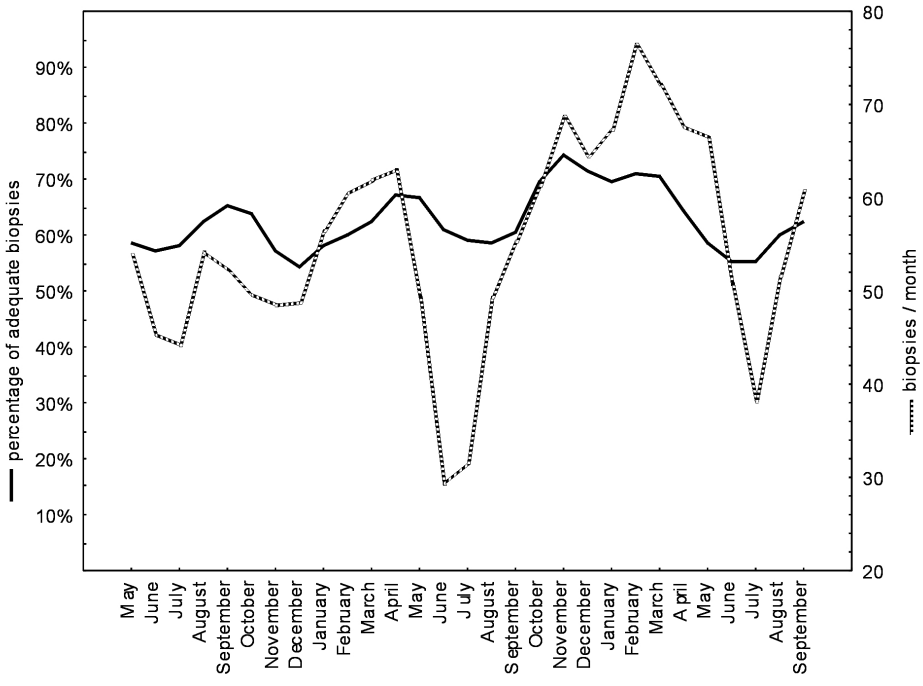

Fig. 4. Adequate biopsy rates in 30 consecutive months (continuous line) plotted along with the number of biopsies in these months (dotted line). The decreases of quality correspond to the three summer holidays within the study period, and to the two ends of a calendar year.

there was one big and one small winter drops in the biopsy quality, both paralleling to the decrease in the amount of biopsies in these periods.

The "Patient" factors

The patients whose biopsies were adequate were on the average significantly younger than these whose trephines were inadequate (mean 51.7 years, SD 15.8 vs. 55.2 years, SD 15.8, $p<0.00001$). It is of note that in the study period we did not receive pediatric biopsies, and the lowest patient age was 16 years. The linear correlation model showed a negative association between the patient's age and the biopsy quality measured as the number of intertrabecular spaces and the length of the interpretable marrow ($r=-0.22$ and -0.16, respectively). Although the correlation coefficients were small, these associations were strongly statistically significant with p values below 0.001. When stratified into the age cohorts the drop in the biopsy quality could

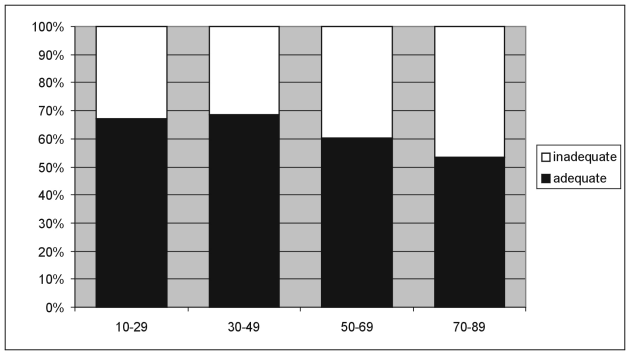

Fig. 5. The percentages of adequate trephine bone marrow biopsies in the consecutive age cohorts.

have been noticed at the age of 50 years (χ^2 p=0.00003). The patient's age did not exert significant influence below 50 years, whereas after this age there was a drop in the biopsy quality in the oldest subjects (p=0.032) (Fig. 5).

Males had slightly better biopsies than females (63.5% vs. 60.3% adequate), but this difference was not significant (χ^2 p=0.14).

The specific type of bone marrow pathology exerted influence on the biopsy quality with χ2 p=0.01. The best biopsies were generally obtained from the patients with chronic myeloproliferative disorders (74% adequate, mean 13.8±7.7 mm), whereas the poorest from the patients with

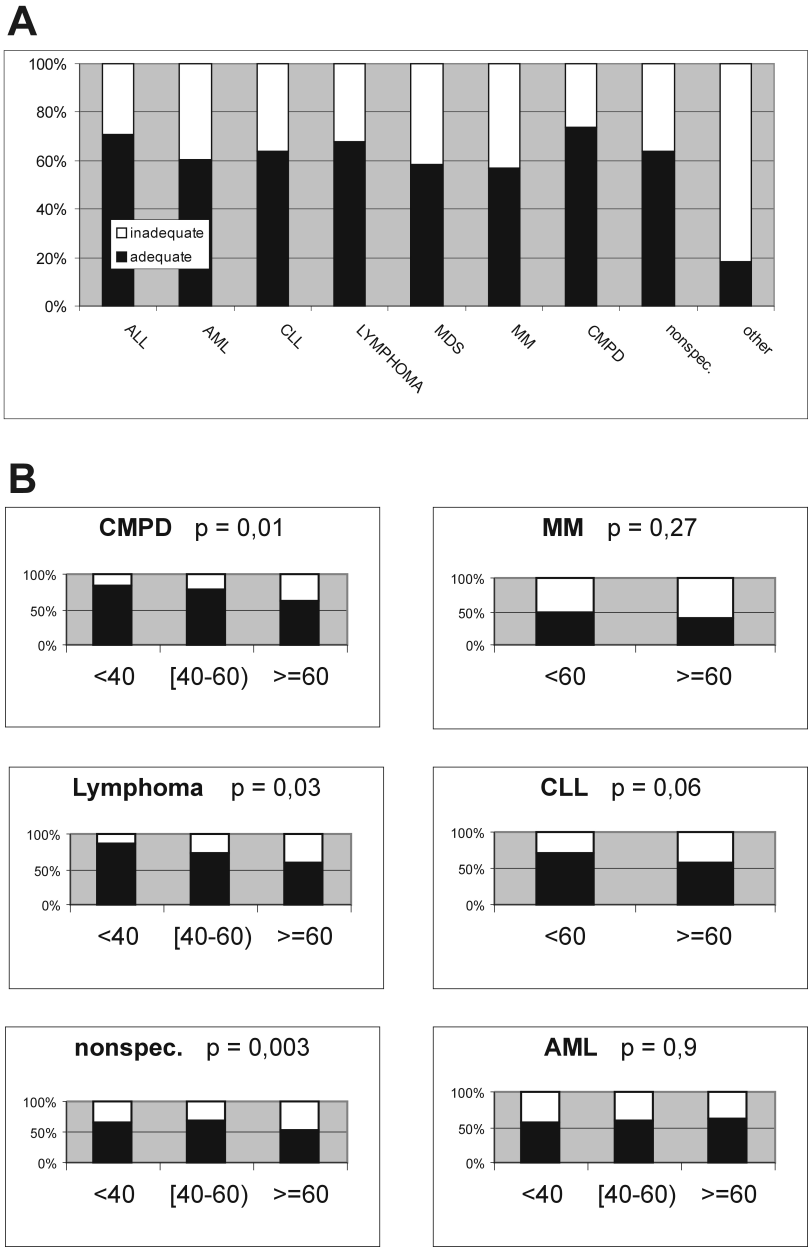

Fig. 6. A. The percentages of adequate trephine bone marrow biopsies against the diagnostic categories (definitions of the categories: see the *Material and Methods*). B. Adequate trephine bone marrow biopsies against patient's age in different diagnostic categories.

a detectable multiple myeloma infiltrate (57% adequate, mean 11.7±6.7 mm) (Fig. 6A). Since the average age within the diagnostic categories varied, sometimes significantly (for instance for ALL 41.4 years, for MDS 62.5, $p=1.2x10^{-9}$), we examined the adequacy of biopsies in the diagnostic categories split into the age cohorts (Fig. 6B). Thus in some diagnostic categories, like AML or MM, the age did not exert any significant influence on the biopsy quality, whereas the age-dependent decrease in the trephine adequacy was observed in older patients with uninvolved marrow (the "nonspecific" category), suffering from CMPD, or with lymphomatous infiltrates.

Among the cases with strong bone marrow fibrosis (+3 or +4) the percentage of successful trephines was significantly higher than in the two other groups (Fig. 7) (χ^2 p=0.03).

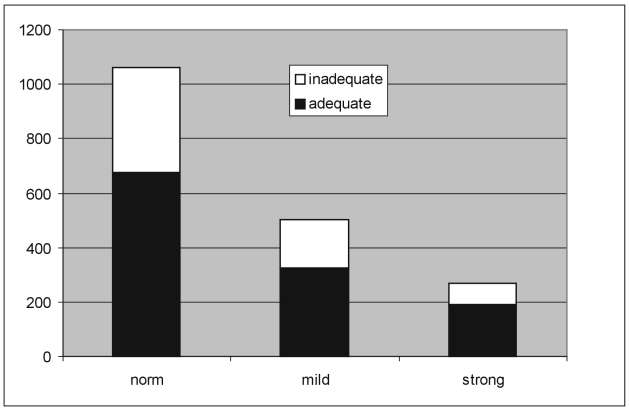

Fig. 7. The association of bone marrow fibrosis and the number of adequate vs. inadequate trephine bone marrow biopsies.

The influence of osteopenia on the trephine adequacy was not easy to examine, as in the extremely poor biopsies it was frequently impossible to assess the quality of bone trabecule. An indirect inference could have been made limiting the analysis to the adequate trephines whose reports always contained the statements on the quality of the bones. The biopsies demonstrating osteopenia were on the average shorter (16.6±7.2 mm) compared to the biopsies with the bone structure evaluated as appropriate for age (17.3±7.4 mm, χ^2 p=0.0034).

To test for the most significant factors influencing the adequacy of trephine bone marrow biopsy we used logistic regression.

The independent factors influencing biopsy adequacy emerging from this analysis were the patient's age (p<0.0001), bone marrow fibrosis (p=0.018), and an operator performing the procedure (p=0.008). Addition of other factors to this model did not improve its goodness of fit.

Twelve members of the EBMWG responded to the questionnaire and submitted the criteria for an adequate trephine bone marrow biopsy adopted in their everyday practice (Table 2).

Discussion

Currently there are no universally adopted criteria for an adequate trephine bone marrow biopsy. The recommendations for a minimal adequate trephine vary between the authors and practitioners. Campbell et al. required at least 5 mm [5]. Brynes et al. considered 15 mm as the minimal

TABLE 2
Numerical criteria for a minimal adequate trephine bone marrow biopsy used in the everyday practice and kindly submitted by 12 members of the European Bone Marrow Working Group to the author's request.

Pathologist	Minimal whole biopsy length (mm)	Minimal bone marrow length (mm)	Minimal number of intertrabecular spaces	Minimal biopsy diameter (mm)
1	15		5	
2	15	12	10	2
3	12	8–10	8–12	2–4
4	20			2
5	20	10		
6	20	20		
7	20	20	5	
8	bilateral 20–30 mm for lymphomas, 10–20 mm for leukemias			
9	20	15	5	1
10	10	5		2
11		20		2
12	20			

length of the specimen [6]. Bain stated that the minimal length was 16 mm, and the ideal over 20 mm after processing [1]. The minimal core length of 20 mm was recommended by the National Cancer Institute – sponsored international working group [7]. Some authors based their criteria on parameters different than the total core length: Schmid and Isaacson suggested at least 5 well-preserved intertrabecular spaces [8], and Coller et al. at least 5 high-power fields [9]. Finally, Roath et al. required a minimal interpretable bone marrow area of 15 mm^2 [10].

All the above guidelines were formulated without the reference to the patient's age. The anatomical and psychological specificity of the trephine bone marrow biopsy in a pediatric setting contributes to bigger problems in obtaining large cores. For neuroblastoma staging the European Neuroblastoma Study Group required at least 5 mm of preserved bone marrow, a criterion that was met by most practitioners [11]. There are no published minimal standards for an adequate trephine in pediatric hematological disorders.

Of these different methods the measurements of the biopsy length seem to be most efficient, being relatively simple and objective. The gold standard would be of course the measurement of the whole interpretable bone marrow area, like proposed by Roath et al. [10]. In practice it is hard to accomplish, as many biopsies (and especially their interpretable parts) are far from even approximate rectangles and such measurements would require sophisticated computer-assisted planimetry or tedious manual counting. Assessing the trephine quality according to its length one has to take into account two important factors. First, it makes a big difference if the core is measured before or after processing. The trephine bone marrow biopsies on histological slides are much shorter than fresh specimens, shrinking by approximately 25%–29% [1, 12]. Second, it has to be clearly stated if the measurement is limited to the preserved bone marrow or encompasses the whole core. The trephine bone marrow biopsy, especially tangential, may contain a surprisingly long chunk of periosteum, bone, cartilage or crushed marrow, which is not always appreciated by a clinician, considering the biopsy to be adequate upon the gross inspection, and not repeating the procedure. The average proportion of these tissues to the interpretable bone marrow part of the cores was like 33% to 67% in a study of Bishop et al. [12].

The published or declared recommendations for an adequate trephine are usually arbitrary, but two studies addressed this issue in a systematic way for specific categories of disorders. Already mentioned study of Campbel et al., based on the examination of 172 patients with diffuse large B-cell lymphoma, showed that the percentage of trephines with neoplastic involvement reached plateau for the cores of 22-30 mm [5]. A group from Manchester examined 767 trephines with a wide spectrum of disorders and found out that the percentage of biopsies with neoplastic infiltrates increased along with the length of interpretable marrow or the total core length to plateau at the values of 8 mm (marrow) and 12 mm (whole core) [12]. According to the latter study our minimal criteria (10 mm of bone marrow after processing or 10 well-preserved intertrabecular spaces) seem quite realistic although most investigators declare that they prefer to adhere to higher standards. This is also illustrated by the results of our questionnaire within the EBMWG (Table 2).

However, in an everyday practice such high standards can only hardly be met. Bishop et al. summarizing the data on the trephine quality in a clinical setting similar to ours in 1992 had the average length of interpretable marrow after processing of only 7.4 mm [12]. In a more recent single-institution study analyzing 84 trephines the whole core length was ranging from 3 to 25 mm (mean 10.7 mm) when the procedure was performed by the doctors, and from 2 to 22 mm (mean 11.0 mm) when the biopsy was done by the trained nurses [13]. The quality of our material is slightly better than in these two British studies, and very similar to the recently published data from Australia [5]. In the material of the Australasian Leukaemia and Lymphoma Group, limited to the biopsies larger than 5 mm, the median whole trephine length was 19 mm [5]. We measured only the bone marrow part of the trephines, but if we assume that this corresponds to 67% of the whole biopsy [12] and similarly limit the analysis to the cores longer than 5 mm (1476 out of 1938 biopsies) we will have an almost identical calculated median length of 20.1 mm.

Although the quality of the trephine bone marrow biopsies in our material is comparable to these published surveys, still only 17.6 % of our biopsies contain more than 20 mm of interpretable marrow, which is a frequently encountered published threshold for a good trephine. Approximately one-third of our biopsies is equal or longer than 15 mm (bone marrow part), which is a median value in the questionnaire filled by the EBMWG members. Thus in realities the quality of trephine bone marrow biopsies may markedly diverge form the declared standards, and in the everyday practice many biopsies are considered inadequate.

The consideration that some doctors make good trephines and some have notorious problems is a trivial one, and is not only our finding [12]. Collecting the data for this study we expected that the practitioners who perform numerous biopsies would have generally better results than the others, which really was the case (Fig. 3). Surprisingly, the outcomes for the group of doctors performing less than one trephine per month was also good, and statistically did not differ from these who made the procedure approximately at least once a week. The intermediate group (20–100 biopsies

in the 30-month study period) was very heterogeneous, containing both very good and very poor operators. This perplexing phenomenon is probably related to the basic mechanisms underlying human learning, when the beginners pay their utmost attention to the challenging task, whereas many of these who already have some, but in fact still limited experience, tend to overestimate it and make errors. The performance of individual operators greatly influenced the outcomes of their hospitals, especially when the number of practitioners doing the trephine bone marrow biopsy was limited.

The number of trephine bone marrow biopsies performed in the area we serve increases more than linearly each year (data not shown), so we expected that this rising trend would also have a positive impact on the biopsy quality. However, the trend line for the biopsy quality was rather erratic, with an insignificant overall rising tendency, and marked drops in quality corresponding to each summer holidays. This paralleled to the decrease in the number of biopsies performed per month at this time, but still can be explained by an increased personal workload for these who stay at work during the vacation time. According to the common practice in July and August the number of stuff is usually close to 50%. Unfortunately the time trends for the individual operators could not have been examined due to relatively small numbers of biopsies per even the most active practitioners in the monthly intervals. Overworking may influence negatively the trephine quality also at the end of the year, when the staff is complete, but traditionally both patients and doctors try to finish the diagnostic process "before Christmas" and before the new budget year, meaning always some insecurity in our country. The pessimistic picture, i.e. that at the level of a whole institution doing more trephines did not necessarily improve their overall quality, emerged also from the Neuroblastoma Study Group report in pediatric patients [14].

Our study differs from the previous audits of the trephine bone marrow quality in looking also at the factors associated with the patient. The strongest factors exerting an impact on the trephine bone marrow biopsy quality were the patient's age and bone marrow fibrosis. Older patients had poorer trephines, which might be at least partially explained by more brittle bones, more frequent obesity and lesser patient's compliance. The "brittle bones" are frequently reported by the clinicians as an obstacle in obtaining a satisfactory core, and the histological equivalent of this phenomenon – osteopenia – was shown in our study to have a significant negative impact on the biopsy quality.

On the other hand the strong bone marrow fibrosis made the procedure easier, an effect opposite to that well known for an aspiration biopsy. This may be explained by more homogeneous consistency of the whole core (analogy to specimen cutting on a microtome is obvious for pathologists here), but also by resistance of fibrotic bone marrow to distortion by a previous aspiration from the same site.

The significant association between the biopsy quality and different diagnostic categories can only partially be explained by the influence of age and fibrosis. For some groups of diseases, like acute myeloid leukemias and multiple myeloma the biopsy quality does not depend on the patient's age, for multiple myeloma being uniformly poor. In multiple myeloma there is no significant difference in the biology of the disease in young people compared to elderly patients [15], so this may at least partially explain the nullification of the age effect on the biopsy quality.

A pathologist's report should always comment on the trephine adequacy, and contain some numerical measure of its quality. Such a feedback is invaluable for the clinicians, and may be used in planning the additional training for these whose performance is poor. An audit of the type we present here identifies not only the operators requiring such training, but also the persons and the centers that potentially can share their good experience.

To summarize, we offer two basic guidelines, which we believe are substantiated by our audit:

1. Not every doctor whose patients require trephine bone marrow biopsy has to do the procedure personally. Ideally the operators should be selected from these who have an occasion to carry out more than one biopsy per week.
2. A trephine bone marrow biopsy is more difficult in some patients than in others, and to some extend this difficulty may be estimated prior to the procedure. A beginner trainee should rather avoid an older lady with multiple myeloma and perform his/her first trephine in a young man with a strong index of suspicion for a chronic myeloproliferative disorder, particularly idiopathic myelofibrosis.

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References

1. *Bain BJ*: Bone marrow trephine biopsy. J Clin Pathol 2001, 54, 737–742.
2. *Bain BJ:* Bone marrow biopsy morbidity and mortality. Br J Haematol 2003, 121, 949–951.
3. *Frisch B, Bartl R:* Biopsy Interpretation of Bone and Bone Marrow. 1st ed. London: Arnold 1999.
4. *Bauermeister DE:* Quantitation of bone marrow reticulin – a normal range. Am J Clin Pathol 1971, 56, 24-31.

5. *Campbell JK, Matthews JP, Seymour JF, Wolf MM, Juneja S; Australasian Leukaemia Lymphoma Group:* Optimum trephine length in the assessment of bone marrow involvement in patients with diffuse large cell lymphoma. Ann Oncol 2003, 14, 273–276.
6. *Brynes RK, McKenna RW, Sundberg RD:* Bone marrow aspiration and trephine biopsy. An approach to thorough study. Am J Clin Pathol 1978, 70, 753–759.
7. *Cheson BD, Horning SJ, Coiffier B, Shipp MA, Fisher RI, Connors JM, Lister TA, Vose J, Grillo-Lopez A, Hagenbeek A, Cabanillas F, Klippensten D, Hiddemann W, Castellino R, Harris NL, Armitage JO, Carter W, Hoppe R, Canellos GP:* Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas. NCI Sponsored International Working Group. J Clin Oncol 1999, 17, 1244–1253.
8. *Schmid C, Isaacson PG:* Bone marrow trephine biopsy in lymphoproliferative disease. J Clin Pathol 1992, 45, 745-750.
9. *Coller BS, Chabner BA, Gralnick HR:* Frequencies and patterns of bone marrow involvement in non-Hodgkin lymphomas: observations on the value of bilateral biopsies. Am J Hematol 1977, 3, 105–119.
10. *Roath S, Smith AG, Choudhury D:* Bone marrow biopsy in non-Hodgkin's lymphoma. J Clin Pathol 1991, 44, 350–351.
11. *Reid MM, Roald B:* Adequacy of bone marrow trephine biopsy specimens in children. J Clin Pathol 1996, 49, 226–229.
12. *Bishop PW, McNally K, Harris M:* Audit of bone marrow trephines. J Clin Pathol 1992, 45, 1105–1108.
13. *Lawson S, Aston S, Baker L, Fegan CD, Milligan DW:* Trained nurses can obtain satisfactory bone marrow aspirates and trephine biopsies. J Clin Pathol 1999, 52, 154–156.
14. *Reid MM, Roald B:* Deterioration in performance in obtaining bone marrow trephine biopsy cores from children. European Neuroblastoma Study Group. J Clin Pathol 1999, 52, 851–852.
15. *Blade J, Kyle RA, Greipp PR:* Multiple myeloma in patients younger than 30 years: report of 10 cases and review of the literature. Arch Intern Med 1996, 156, 1463–1468.

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