Bone Marrow Aspiration: A Randomized Controlled Trial Assessing the Quality of Bone Marrow Specimens Using Slow and Rapid Aspiration Techniques and Evaluating Pain Intensity

Mette Grønkjær^a Connie F. Hasselgren^b Anne Sofie L. Østergaard^b
Preben Johansen^c June Korup^b Martin Bøgsted^{b,e} Anders E. Bilgrau^{b,d}
Paw Jensen^b

^aClinical Nursing Research Unit, and Departments of ^bHematology and ^cPathology, Aalborg University Hospital, and Departments of ^dMathematical Sciences and ^eClinical Medicine, Aalborg University, Aalborg, Denmark

Key Words

Bone marrow aspiration · Bone marrow quality · Pain intensity

Abstract

Background/Aims: Bone marrow aspiration (BMA) is an essential procedure in the examination of hematological disorders, but there is limited evidence as to whether the aspiration rate affects specimen quality. We aimed to assess the specimen quality and pain intensity using slow (S-technique) or rapid (R-technique) aspiration. **Methods:** This was a single-center, prospective, randomized patient- and assessor-blinded study of 482 patients scheduled for BMA. Specimen quality was evaluated by grading bone marrow (BM) cellularity and counting the number of marrow particles. Pain was assessed using a visual analog scale (VAS). **Results:** We found a significant difference between the 2 groups with regard to the quality of specimens. For cellularity, the odds ratio (OR) for having a poor quality aspirate using the S-technique versus the R-technique was 3.05 [confidence interval (CI) 1.79–5.31]. For BM particles, the quality of specimens with the S-technique proved to be poor compared with the R-technique (OR 2.52; CI 1.51–4.28). We found a statistically significant difference of 1 VAS point ($p < 0.001$) of the median pain intensity in favor of the S-technique. **Conclusion:** Even though the pain intensity is significantly higher with the R-technique, the median difference is relatively small. We propose that the R-technique is preferable to the S-technique due to better specimen quality.

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Introduction

Bone marrow aspiration/biopsy (BMA) is an essential procedure in the initial examination and during the course of hematological disorders. Obtaining the optimal quality of the specimen for pathologic review is obviously important [1]. Textbooks and BMA guidelines rarely mention the aspiration technique, and when they do, rapid aspiration is mentioned [2–4]. Former studies have examined the impact that biopsy devices, operator profession and experience have on the quality of bone marrow (BM) assessment and pain [1, 5–10]. To our knowledge, studies on the aspiration technique and its association with spec-

imen quality are sparse. A randomized, cross-over trial aimed to detect a technique that reduces pain without loss of specimen quality indicated that BMA with low differential pressure tended to be less painful, while maintaining specimen quality [11]. However, that study comprised only a few patients ($n = 49$) and the study design details are not clear as it is only published as an abstract. Another study concluded that the first 2.5 ml of BM aspirated from one puncture site generated the best specimen quality, but did not consider the aspiration rate [12]. It is well known that BMA is associated with pain and discomfort [2, 13–18]. One study evaluated pain in cancer patients undergoing different types of invasive examinations and found that the highest pain levels were related to procedures such as BMA [19]. A prospective study on procedure-related pain among adult hematologic patients who underwent BMA found that 70% reported pain during BMA; 35% of these reported severe-to-worst-possible pain [14]. Several independent factors are associated with BMA-induced pain such as young age, insufficient information prior to the procedure, previous BMA experience, anticipatory anxiety, long duration and the difficulty of the procedure [15, 20–21]. A recent literature review on strategies for pain reduction during BMA highlighted that research so far has concentrated on pain-associated factors rather than pain-reducing measures [22]. A study assessed pain and anxiety during BMA among patients versus health care professionals and found that registered nurses and physicians underestimated severe pain as well as anxiety for BMA outcome and needle insertion [13]. For patient comfort purposes, it is stressed that patients must be warned of the possibility of suction pain before it occurs and be reassured that it will be brief [23]. It is not known if the intensity of pain is dependent on the aspiration technique. Hence, this study aimed to assess the quality of BM specimens and the overall pain intensity using slow and rapid aspiration techniques. We hypothesized that the proportion of poor-quality specimen samples with the slow aspiration technique (S-technique) is no worse than that of the rapid (R)-technique (primary outcome). In addition, we hypothesized that there is no difference in pain intensity when using the S- and R-techniques (secondary outcome).

Material and Methods

Study Design and Patients

In this single-center, prospective, patient- and assessor-blinded study, 482 adult patients at the Department of Hematology, Aalborg University Hospital, Denmark, were randomized for either

slow aspiration (S-technique) or rapid aspiration (R-technique). Data were collected from November 2012 to September 2013. Patients eligible for inclusion were >18 years old and scheduled for BMA. Patients could only be enrolled once. Excluded from participation were patients with altered consciousness, those not able to speak and understand Danish, and those with an international normalized ratio (INR) >3.5 .

The sample size was based on power calculations using data from a pilot study of 60 consecutive standard BMAs (rapid aspiration) examined for cellularity and the number of marrow particles (where 'no' or 'few' nucleated cells and strictly <11 marrow particles were defined as inferior quality). The proportion of accepted biopsies using standard rapid aspiration was 75% [confidence interval (CI) 64–86] for cellularity and 78.3% (CI 67–89) for the number of marrow particles. The power calculations used marrow particles as a measure of quality due to the high success rate. We conducted a noninferiority test for the null hypothesis that slow aspiration provides poorer specimen quality than rapid aspiration. Based on the proportion of accepted biopsies, the noninferiority margin was 10%. With a 5% significance level, a minimum of 241 patients in each aspiration group was needed to reach a power of 80% for the null hypothesis of no difference in marrow quality.

Data Collection

The study was integrated in the department's ordinary BMA practice by inviting patients to participate as part of their scheduled BMA. At the Department of Hematology, Aalborg University Hospital, BMAs are conducted by 5 registered nurses (RNs), who have advanced training and several years of experience in performing BMA. Upon informed consent, patients were randomized for either the S-technique or the R-technique via a numbered envelope.

Intervention

Aspiration of BM was performed using either the S-technique (a slow, low, differential pressure, uniform pull of a 10-ml syringe and aspirating in the same pace as the appearance of the BM for approx. 5–15 s) or the R-technique (a quick pull creating a high differential pressure in a 10-ml syringe for approx. 1 s and holding a high differential pressure during the aspiration of the marrow). Local anesthesia was used for every patient. The biopsy site was the posterior superior iliac crest. The aspiration technique was blinded for the patient and the pathologist and only known to the RN conducting the BMA. Aspiration and biopsy were performed for all patients. Apart from the difference in aspiration technique, the BMA was performed according to the standard procedure of the department. This involved 2 aspirations from the same puncture site followed by biopsy from another puncture site. From each aspiration, 1.5–3 ml of BM was collected for cytologic preparation, flow cytometry, cytogenetics (upon indication) and bio-banking.

Outcome

The primary outcome measure was the BM specimen quality. All samples were examined by a single, experienced pathologist who assessed the quality of the specimen from the first aspiration. BM cellularity was graded as either 'no', 'few', 'moderate' or 'many' BM cells. We defined no or few BM cells as inferior quality. The number of marrow particles was graded as 0–25 and >25 . We defined <11 marrow particles as inferior quality. The secondary outcome measure was the patient's pain intensity. Immediately after

BMA, patients were asked to assess the overall pain intensity according to a visual analog scale (VAS) which described the patient's pain intensity from 0 to 10 (0 = no pain at all and 10 = worst pain imaginable). Preprocedural analgesia or anxiolytics were not routinely used.

Statistical Analysis

Comparisons between groups of observations were carried out using Fisher's exact test for categorical variables and Wilcoxon's rank-sum test for continuous variables at a 5% significance level. All effect estimates are provided with 95% one-sided CIs. The analysis of cellularity and BM particles were performed as noninferiority (one-sided) tests for the null hypothesis that the proportion of inferior quality aspirates of the S-technique is no worse than that of the R-technique.

We collected data on diagnoses and grouped the patients into 6 groups: (1) acute leukemia and myelodysplastic syndrome (AL), (2) multiple myeloma (MM), (3) malignant lymphoma and chronic lymphocytic leukemia (MCLL), (4) myeloproliferative disorders (MPD, mastocytosis excluded), (5) other (mainly monoclonal gammopathy of undetermined significance, but also mastocytosis, hemophagocytosis and BM infiltration from other cancer diseases) and (6) normal BM (N). Subanalyses stratified on disease groups were carried out. Analysis of variance (ANOVA) was used to study the association between BM quality and pain and the effects of the explanatory variables. In particular, multiple logistic regression was used to model the association between cellularity and marrow particles and the covariates aspiration technique, disease group, their interaction and RN. Multiple linear regressions were used to model the association between VAS pain intensity and the covariates with aspiration technique, disease, operator, age and sex.

Ethics

The study was approved by and conducted in compliance with the North Denmark Region Committee on Health Research Ethics (approval No. N-20120058). It was performed according to the Declaration of Helsinki and written informed consent was obtained from all patients before enrolment.

Results

Patient Characteristics

A total of 482 patients participated in the study (table 1). There were no significant age and gender difference between the 2 aspiration groups. Of the study population, 11 patients received anxiolytics as premedication (4 in the slow-aspiration group and 7 in the rapid-aspiration group). No patients received analgesia as premedication. Patients were allowed to take their usual medicine. In fibrotic marrow specimens it is difficult to estimate cellularity or count the number of marrow particles, so some of these were subsequently excluded. We included BM fibrosis (MF) grade 0–1 and excluded samples with MF grade 2–3 (using the WHO grading criteria for MF) [24]. Prior to analysis, 38 patients were excluded due to

Table 1. Overview of patient characteristics

Characteristics	Aspiration		
	slow (n = 218)	rapid (n = 226)	total
Gender			
Female	79 (17.8)	103 (23.2)	182 (41)
Male	139 (31.3)	123 (27.7)	262 (59)
Age, years	64.71 ± 14.11	64.79 ± 13.62	64.75 ± 13.85
Age group			
18–40 years	13 (2.9)	12 (2.7)	25 (5.6)
41–50 years	23 (5.2)	23 (5.2)	46 (10.4)
51–65 years	64 (14.4)	73 (16.4)	137 (30.9)
66–80 years	95 (21.4)	94 (21.2)	189 (42.6)
>80 years	23 (5.2)	24 (5.4)	47 (10.6)
BM cellularity			
0	9 (2)	1 (0.2)	10 (2.3)
1+	51 (11.5)	24 (5.4)	75 (16.9)
2+	87 (19.6)	57 (12.8)	144 (32.4)
3+	71 (16)	144 (32.4)	215 (48.4)
BM particles			
0–10	59 (13.3)	29 (6.5)	88 (19.8)
11–25+	159 (35.8)	197 (44.4)	356 (80.2)
Sample usable			
Yes	214 (48.2)	225 (50.7)	439 (98.9)
No	4 (0.9)	1 (0.2)	5 (1.1)
Dry BM			
Yes	0	3 (0.7)	3 (0.7)
No	218 (49.1)	223 (50.2)	441 (99.3)
BM infiltration			
Yes	42 (9.5)	31 (7)	73 (16.4)
No	176 (39.6)	195 (43.9)	371 (83.6)
Diagnosis class			
AL	17 (3.8)	15 (3.4)	32 (7.2)
Other	10 (2.3)	19 (4.3)	29 (6.5)
MCLL	32 (7.2)	37 (8.4)	69 (15.6)
MM	21 (4.7)	10 (2.3)	31 (7)
MPD	20 (4.5)	18 (4.1)	38 (8.6)
MPD and MCLL	0	1 (0.2)	1 (0.2)
N	117 (26.4)	126 (28.4)	243 (54.9)
Values express n (%) or mean ± SD.			

hypoplasia/aplasia (n = 5), fibrosis (n = 13), samples lost/destroyed due to an error during tissue processing at the Department of Pathology (n = 10), hairy-cell leukemia (dry tap; n = 3), BM necrosis (n = 2), osteosclerotic metastatic carcinoma (n = 2), information lost (n = 2) and other biopsy needles used (n = 1; fig. 1).

Fig. 1. Flowchart of the study.

Fig. 2. Observed frequencies of BM particles and cellularity for the S- and R-techniques.

Bone Marrow Quality

We found a significant difference in the quality of the specimen between aspiration techniques. For BM cellularity, the odds ratio (OR) for having a poor-quality aspirate using the S-technique compared to the R-technique was 3.05 (CI 1.79–5.31, $p < 0.001$; fig. 2; table 2). For marrow particles, the OR for having a poor-quality aspirate using the S- versus the R-technique was 2.52 (CI 1.51–4.28, $p <$

0.001; table 2). The pathologist assessed whether the specimen as a whole (including blood, biopsy and aspirated marrow) was usable. For this question, there was no statistically significant difference (OR 4.19, $p = 0.208$) between the 2 groups, although there was a trend towards better quality using the R-technique (table 2). According to the disease groups, the significant differences were only retained in the MCLL and N groups (table 2). From the

Fig. 3. Box plots of the VAS pain score for the S- and R-techniques.

logistic regression and corresponding ANOVA (online suppl. table S2; for all online suppl. material, see www.karger.com/doi/10.1159/000438480), only the aspiration technique was significant for both measures of sample quality (particles: $p < 0.001$ and cellularity: $p < 0.001$). The RN effect was significant only for the BM particles ($p = 0.03$). There was no significant effect of disease group or interaction between disease group and aspiration technique (online suppl. table S1). The significant RN effect prompted further investigation in terms of RN experience. The 5 RNs were categorized as having more or less experience at the time of the aspiration from the date of employment. The level of experience was defined by a threshold of 3 years. The RNs have all approximately been performing 6 aspirations per day once a week since the date of employment. By Fisher's exact test, the experience level of the RN was independent of the sample quality by both measures (cellularity: $p = 0.901$ and BM particles: $p = 0.806$).

Pain Intensity

The median VAS was 2 and 3, using the S-technique and R-technique, respectively (fig. 3). The difference of 1

Table 2. Fisher's exact test for BM particles and cellularity for all patients, stratified according to disease group

Response		Odds		OR	95% CI	p value
		slow	rapid			
<i>All patients</i>						
Particles		0.37	0.15	2.515	1.51–4.28	<0.001
Cellularity		0.38	0.12	3.046	1.79–5.31	<0.001
Nonusable sample		0.02	0	4.193	0.41–207.81	0.208
<i>Disease group</i>						
AL	Particles	0.55	0.25	2.129	0.35–16.45	0.444
	Cellularity	0.7	0.36	1.886	0.35–11.67	0.472
MCLL	Particles	0.68	0.19	3.579	1.06–13.55	0.030
	Cellularity	0.39	0.09	4.467	0.98–28.39	0.053
MM	Particles	0.17	0.11	1.482	0.1–87.33	1
	Cellularity	0.4	0	Inf	0.61–Inf	0.141
MPD	Particles	0.33	0.27	1.243	0.22–7.59	1
	Cellularity	0.43	0.27	1.588	0.3–9.38	0.716
N	Particles	0.33	0.12	2.626	1.26–5.72	0.007
	Cellularity	0.33	0.12	2.626	1.26–5.72	0.007
Other	Particles	0.25	0.12	2.066	0.13–33.44	0.592
	Cellularity	0.25	0.06	4.249	0.19–279.29	0.267
Inf = Infinity.						

Table 3. Median VAS pain intensity and Wilcoxon's rank sum test for all patients and stratified into disease groups

	Median VAS		p value
	slow	rapid	
All patients	2	3	<0.001
<i>Disease group</i>			
AL	2	3.5	0.173
Other	1	4	0.001
MCLL	2	3	0.016
MM	3	3.5	0.047
MPD	2.5	4	0.038
N	2	3	<0.001

VAS point between the S- and R-techniques in median pain intensity was small, but was statistically significant ($p < 0.001$) in favor of the S-technique (table 3).

The statistical significance was retained in all but the AL patient subgroup (table 3). The ANOVA analysis also yielded strong evidence for an effect of the aspiration technique ($p < 0.001$). Here, we found mild evidence ($p = 0.044$) that VAS pain decreases with age (online suppl. table S2). Furthermore, the data are consistent with no

effect of disease group, RN and gender on the VAS pain intensity as well as no interacting effect between disease group and aspiration technique (online suppl. table S2).

Discussion

From the measurements of the BM cellularity and particles, we found evidence that the R-technique generated a better specimen quality than the S-technique. Pain intensity was significantly higher when using the R-technique compared to the S-technique. The increase of 1 in median VAS pain intensity, however, can be considered small compared to the variability of the VAS in each group (fig. 2). Analysis according to disease group showed the same tendency as for the overall analysis, but was only significant in the MCLL and N groups. The nonsignificance of the remaining groups may be a matter of low power due to small subgroups. Similarly, the ANOVA analysis also suggested that the impact of the aspiration technique on sample quality is unchanged within each disease group. In other words, the effect of the aspiration technique was independent of the disease status. The exclusion of MF grade 2–3 may potentially bias the subgroup of MPD. As the results regarding BM quality and pain from this subgroup were in line with the other subgroups, we believe the potential bias was minimal and had no impact on the overall results.

We found a clear difference in median pain across patients. This observation is in agreement with the Japanese study in which BMA with low-differential pressure tended to be less painful [11]. The same study, however, indicated that slow suctioning did not affect the quality of the specimen, which is not supported by our study. This inconsistency may be due to a fairly small sample size in the Japanese study. Although the pain intensity was higher among patients in the R-technique group, the relatively small difference of 1 on the VAS convinced us to attribute more importance to specimen quality than pain intensity. It is, however, important to acknowledge that all patients are different and that pain is a subjective phenomenon. Moreover, patients with hematological disorders often have repeated BMAs to determine their disease trajectory, treatment efficacy and recovery process. Former studies show a clear association between pain intensity, previous BMAs and inadequate information [16, 20, 25]. In patients who require repeated BMAs, fear and anticipatory pain for this procedure can cause great distress [26]. The multitude of factors besides the aspiration technique involved in the perceived pain intensity highlights the im-

portance of taking the individual into consideration before and during BMA.

For this study, we included all eligible adult patients who were scheduled for a BMA. The aim was to conduct a study that was as identical to the daily clinical work as possible. The BMA procedure at Aalborg University Hospital is performed by 5 RNs with advanced training. The feasibility of the study was dependent on the daily practice of the department including staffing and work schedules, and thus all 5 RNs were involved in the data collection. They were trained to follow the same procedure when informing the patients, obtaining consent and performing the randomization dependent on BMA. For practical reasons, it was not possible to ensure blinding of the RN who asked the standardized questions. Although we have ensured uniform information, BMA procedure and pain assessment, we cannot rule out the risk of potential bias caused by minor differences between the RNs. The blinding was complete for the pathologist regarding the evaluation of the quality parameters. For the pain assessment, the patients were blinded for the aspiration technique when scoring the intensity of pain using the VAS. The use of VAS for assessing pain intensity can be questioned as it is predominantly used for comparing pain assessment in individuals rather than between groups, which creates a potential bias regarding the measurement of pain. However, the absence of appropriate pain assessment scales, the frequent use of VAS in similar studies and the large sample size justify the use of VAS in this setting [27]. The fact that our study included more men than women may be owing to the fact that more men than women suffer from hematological illness [28]. We do not anticipate that gender influences specimen quality. Seeing that there was no difference on pain measurement between men and women, we believe that gender imbalance does not affect the main conclusions. We acknowledge that the single-center study design is a weakness that may influence the external validity of our findings. Widespread changes in clinical practice generally require results from multi-center studies in order to validate the results in different settings.

In conclusion, the quality of BMA using the R-technique for aspirating BM is better than when using the S-technique. The pain intensity is significantly higher when using the R-technique than with the S-technique. However, the difference in pain intensity is only moderate compared to the general variability of the VAS pain score. Even though there is a difference in pain intensity, we believe that preference should be given to the R-technique in BMA due to the better specimen quality.

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Disclosure Statement

All authors declare that there were no conflicts of interest.

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