ORIGINAL ARTICLE

A comparison of two techniques of preparing bone marrow aspirate slides

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Abstract

Objective: To compare direct smear technique with ethylenediaminetetraacetic acid (EDTA) preserved smear technique in terms of preparing bone marrow aspirate slides.

Methods: This prospective study was carried out between September 2009 and July 2012 at the Haematology/Oncology Department, King Khalid University Hospital, Riyadh, Saudi Arabia. With a standard gauge disposable bone marrow aspirate needle, 0.5 to 1.0 ml bone marrow was aspirated with a 10ml syringe. Half of the marrow was immediately transferred to an EDTA tube with gentle mixing, while slides were prepared directly from the rest of the sample in the syringe. The tube sample was used to prepare slides at the end of the procedure. A score of 1-4 was assigned to each slide depending on the quality and number of particles.

Results: A total of 245 bone marrow aspirate samples were evaluated related to 216 patients. Of the total, 238 (97%) samples were included in the study. The mean score for the direct smear group was 3.40±0.79 and for the EDTA smear group it was 3.34±0.75 (p=0.27), which was not statistically significant. An informal comparison of the morphological analysis of the samples did not reveal any differences.

Conclusion: Bone marrow aspirate slides prepared at the end of the procedure from EDTA preserved samples were not inferior to slides prepared directly from the aspirated sample.

Keywords: Bone marrow aspirate, Direct smear, EDTA smear. (JPMA 66: 528; 2016)

Introduction

Bone marrow aspirate (BMA) is an indispensable adjunct to the study of many benign and malignant haematological and systemic diseases, and may be the only way in which a correct diagnosis can be made.[1] BMA is usually performed in the posterior iliac crest under local anaesthesia. A good, particulate BMA sample is needed for optimal morphological interpretation and accurate diagnosis. Generally, the first aspirated sample is reserved for morphological examination, as it is likely to be the best sample containing BM particles. The subsequent aspirations, if needed for cytogenetics and immunophenotyping, are likely to become haemodilute. A number of factors can affect the quality of BMA smears, including the expertise of the performer, BM status (e.g. fibrosis) and the method of specimen handling. The latter can influence quality of the specimen during different steps such as storage, spreading and staining.

Proper spreading of the BMA on the glass slides is essential for optimum evaluation and interpretation. BMA smears are usually made directly from the non-anti-coagulated aspirate (direct smear, conventional method), but many laboratories now prepare anti-coagulated aspirate samples using ethylenediaminetetraacetic acid (EDTA) tubes. The conventional method can eliminate some of the post-collection variables such as dilutional effect due to excess of anti-coagulant and storage artefacts.[1] However, it is inconvenient as the operator has to rush to prepare the slides which may lead to poor quality of BMA smears, particularly if the performer is not fully proficient. The use of anti-coagulated aspirate improves the quality of smears as the slides can be prepared in a relaxed manner at the end of the procedure. In addition, it minimises the risk of clotting and enables the preparation of multiple slides needed in conditions like acute leukaemia. Possible disadvantages of EDTA storage include dilutional effect due to excess of anti-coagulant or morphological artefacts on prolonged storage.[1-3]

International Committee for Standards in Haematology (ICSH) guidelines for the standardisation of BM specimens and reports recommend that BMA slides should be prepared by direct technique and additional BMA sample can be stored in the EDTA tube to make further slides.[4] The two techniques of preparing the BMA smears, i.e., direct smear and EDTA-preserved smears, have not been directly compared in a prospective manner. Our practice suggested that preserving BMA samples in an EDTA tube for a few minutes does not cause any significant dilutional

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effect or storage artefacts. Thus, if the method of preparing slides with the EDTA-preserved BMA at the end of the procedure is not inferior to the conventional method of preparing the slides directly, it would be more convenient for the operator, particularly the trainee staff. It would also be more practical in cases where BMA sample may clot rapidly and in conditions like acute leukaemia where a number of slides need to be prepared.

As BMA is an important procedure to diagnose a variety of benign and malignant haematological disorders.[5-11] optimal methods to prepare BMA slides need to be studied. Until recently, there were no studies comparing different methods of preparing BMA slides.[12-14] We conducted this study to compare the two techniques of preparing BMA slides.

Patients and Methods

This prospective study was carried out between September 2009 and July 2012 at the Haematology/Oncology Department, King Khalid University Hospital, Riyadh, Saudi Arabia. Patients were subjected to BMA procedure according to the standard indications for investigation of haematological or systemic disease.[1-4] The study was approved by the institutional review board and written informed consent was obtained from all patients. BMA was performed in the posterior iliac crest in the lateral decubitus position as described in literature.[3-5] Posterior iliac crest site was identified by palpation, and the overlying skin was prepared with an antiseptic. The skin, subcutaneous tissue and the periosteum, were infiltrated with 2% lignocaine for local anaesthesia, and younger patients received sedation with Midazolam, 5-10mg intravenously (IV), which was reversed with flumazenil after the procedure. In a few cases the procedure had to be performed in the anterior iliac spine because of obesity and difficulty to identify the posterior iliac crest.

A standard-gauge disposable BMA needle was used and 0.5 to 1.0 ml bone marrow was aspirated with a 10 ml syringe. Half of the marrow was immediately transferred to an EDTA tube with gentle mixing, while slides were prepared directly from the rest of the sample in the syringe.

Most of the procedures and spreading of slides were performed by the junior doctors (Registrars and Residents), which is the usual practice in many tertiary care teaching centres. We did not have a dedicated technician to perform the procedure or spreading of slides. Operators had attended at least a few sessions with a senior operator and had performed multiple BMA procedures independently before doing the procedure for the study. At the end of the procedure, slides were prepared from the EDTA tube sample. Although, many more procedures were performed during this period, the samples were not included if an operator reasonably proficient to spread the slides was not available or the time lapse between the aspiration and the spreading was more than 30 minutes.

For comparison, a scoring system was devised which ranged from 1-4 (Table-1). In the first step, samples were scored on gross appearance of the slides depending upon the quality and particles present. Scoring was done by the operator as well as by an independent observer for more objective evaluation.

Slides were stained with the May-Grunwald-Giemsa method and examined microscopically. The assessment of the bone marrow was made by a haematopathologist according to the generally accepted standards.[1-5] Along with the standard evaluation and reporting of the BMA for each sample, an informal comparison of the morphological analysis of the slides prepared with the direct and indirect method was made. Microscopic evaluation and comparison was performed by experienced haematopathologists. Because of the technical issues and difficulty in standardisation, only slides prepared with May-Grünwald-Giemsa (MGG) staining were compared and slides for cytochemistry and iron staining were not compared.

Data were analysed using SPSS, version 20. Comparison between the two groups was made with paired t-test and a p-value of $\leq$0.05 was considered significant.

Results

A total of 245 BMA samples were evaluated related to 216 patients. Seven (2.87%) samples were excluded; 3 (43%) because of faulty preparation, and 4 (57%) were clotted samples. The patient population consisted of a wide variety of diagnoses or suspected diagnoses managed in a tertiary care centre (Table-2). The median time period between the aspiration of BM and the slide preparation from EDTA samples was 14mts (range: 5-30mts).

The mean score for the direct smear group was 3.40±0.79 and for the EDTA smear group it was 3.34±0.75 (p=0.27). There was a slight trend towards the direct smear being better, but

Table-1: Scoring system according to the quality of bone marrow aspirate (BMA).

Score	BMA quality
1	Dry tap
2	Smear without particles
3	Smear with up to 3 particles
4	Smear with multiple particles

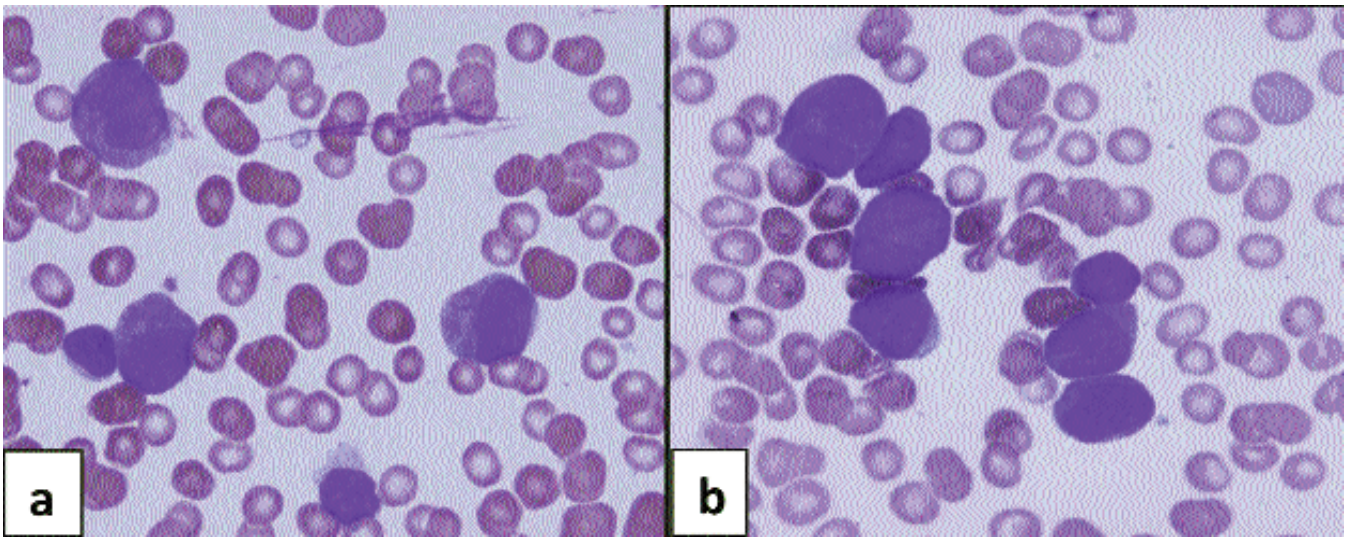

Figure: Comparison of diagnostic interpretation of ethylenediaminetetraacetic acid (EDTA) and direct smears. Bone marrow aspirate (BMA) from a case of acute promyelocytic leukaemia (APL) shows no morphological differences between EDTA smear (a), and direct smear (b).

Table-2: Indications for bone marrow (BM) examination, type of diagnoses or suspected diagnoses and the number of patients in each category.

Diagnoses and indications to perform BM examination (%)	Number
Lymphoma or suspected lymphoma	50
Workup of cytopenias	30
AML/MDS	30/3
CML/MPD	16/12
Mutiple myeloma	19
ALL	15
Immune thrombocytopenia (ITP)	13
Anaemia investigation	10
SLE/RA	9/3
CLL	9
Tuberculosis/PUO	5/2
Others	19

AML: Acute myeloid leukaemia
MDS: Myelodysplastic syndrome
CML: Chronic myeloid leukaemia
MPD: Myeloproliferative disorder
ALL: Acute lymphoblastic leukaemia
SLE: System lupus erythematossus
RA: Rheumatoid arthritis
CLL: Chronic lymphocyte leukaemia
PUO: Pyrexia of uncertain origin.

this difference was not statistically significant in the overall interpretation of the direct and EDTA smears (Table-3).

An informal comparison of the morphological analysis and diagnostic interpretation of direct smears and EDTA smears by the haematopathologists did not reveal any notable differences in the two types of smears (Figure).

Table-3: Patients characteristics and results.

Characteristic	Value
Age in years (median, range)	38, 14-91
Gender (male/female)	121/95
Direct smear score (mean±SD)	3.40 ± 0.79
EDTA smear score (mean±SD)	3.34 ± 0.75
Confidence intervals	-.0486 to .1731
P-value	0.27

SD: Standard deviation
EDTA: Ethylenediaminetetraacetic acid.

Discussion

This study showed that BMA smears prepared from the samples preserved in EDTA were not inferior to direct smears and a few minutes' delay in making the smears did not cause any significant artefacts. The preservation of BMA in EDTA was particularly useful in cases of acute leukaemia, where a lot of slides are needed for cytochemistry and the aspirate may clot quickly. It had the additional advantage of allowing the making of smears leisurely at the end of the procedure. In addition, the diagnostic yield of direct smears and EDTA smears was not different, indicating that EDTA smears are comparable in quality for the diagnostic interpretation of BMA samples. This is reassuring, particularly for the trainee staff, as they do not need to rush to make direct smears, but can save part of the aspirate in the EDTA tube and make smears from it at the end of the procedure.

The International Committee for Standards in Haematology (ICSH) proposed guidelines for the standardisation of BM specimens and reports in 2008.[4] On reviewing the literature, we found that there is limited published evidence regarding BMA procedure, particularly the technique of preparing the slides, and most of the references cited are based on personal experience and expert opinions.[4] DiFrancesco et al. reported the first study on comparison of direct smear and EDTA smear.[12] They had similar results to our study and concluded that EDTA smears are not inferior to the direct smears although this was a retrospective and smaller study comparing samples from 3 different sites. Lewandowski et al. recently reported comparison of the wedge-spread and the crush techniques.[13,14] They compared the two techniques in healthy subjects and in patients with malignant disorders of haematopoiesis. Because the crush preparations can only be made in particulate samples, they only analysed BMA samples containing particles. Although they showed the superiority of crushed technique, but this method is applicable only to particulate samples, while in routine practice it is not possible to obtain particulate samples in all cases and evaluation has to be made on whatever material is available.

Storage effect in blood cell morphology starts to appear within hours of bone marrow collection. However, previous experience and our practice suggest that smears made from BMA which has been standing for less than 1 hour, are indistinguishable from smears made immediately after collection[2] (Figure). Wang et al. showed that bone marrows which were stained within 24 hours did not show marked discernible dyserythropoiesis.[15] However, after one day of storage, sufficient dysplastic changes occurred to cause difficulty in the interpretation of BMA. In order to avoid the storage effect, we prepared all our smears within a few minutes of aspiration.

Our study has certain limitations. Samples were taken and slides were made by the bedside by multiple operators of variable proficiency as there was no dedicated expert technician available. This may have introduced heterogeneity in the samples and affected the quality of the samples and results. In addition, evaluation of the samples was made without blinding which may have introduced bias in the scoring. Nevertheless, our technique and procedure used in the study are close to day-to-day real practice in most of the centres, which is an indirect strength of this study.

Conclusion

BMA slides prepared at the end of the procedure from EDTA-preserved samples were not inferior to slides directly prepared from the aspirated sample. This is reassuring and has the advantage of preparing multiple slides at the end of the procedure in a relaxed manner.

Acknowledgement

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