ORIGINAL RESEARCH

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Bone marrow aspirate collection and preparation – A comparison of three methods

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Abstract

Purpose: Preparing bone marrow smears using non-anticoagulated bone marrow aspirate is a traditional practice but many laboratories now use anticoagulated aspirate samples in K-EDTA. There are no published studies comparing the effectiveness of these two methods. This report compares the readability of slides, prepared using non-anticoagulated and anticoagulated methods, from three laboratories in Hamilton Ontario.

Methods: A blinded set of 129 aspirate slides prepared using anticoagulated and non-anticoagulated methodologies (using K-EDTA) was reviewed by three reviewers. Slides were classified as unreadable if two of the three observers rejected them based on a standardized survey.

Results: The proportion of slides classed as unreadable varied widely (5.0% to 46.9%) depending on collection and slide preparation methods. Degree of coagulation did not affect readability.

Conclusion: A measurable advantage to using non-anticoagulated bone marrow was not demonstrated. Immediate anticoagulation of bone marrow samples, with laboratory personnel at the bedside to assess sample quality, followed by slide preparation in the laboratory provided the best results.

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Making bone marrow smears on non-anticoagulated bone marrow aspirate is traditional practice but many laboratories now prepare anticoagulate aspirate samples using K-EDTA [1-5]. The use of anticoagulated aspirate has several advantages: it is more convenient; it improves the likelihood of good quality bone marrow smears by eliminating clotting; and it reduces the biohazard risks caused by preparation of aspirate slides in a patient's room. A disadvantage of anticoagulation is it may cause morphologic artifacts if an excess of EDTA is used [6].

Our program employs three methods to collect and prepare bone marrow aspirate samples. Two employ K-EDTA anticoagulation and one uses non-anticoagulated aspirate. To date, there are no published studies comparing the effectiveness of anticoagulated and non-anticoagulated methods. This report summarizes the results of our comparison of the three techniques; conducted to determine the best procedure for standardization within our regional laboratory program.

Materials and Methods

A blinded set of 129 bone marrow aspirate slides was assembled, in which a minimum of 40 consecutive retrospective case slides were prepared by each method. Site and methods were as follows: Juravinski Hospital and Cancer Centre (JHCC) : non-anticoagulated aspirate collected on a slide and smears made at the bedside by laboratory personnel; McMaster University Medical Centre (MUMC): aspirate collected by ward staff into a K-EDTA Vacutainer (BD Diagnostics, Franklin Lakes, NJ) and smears made in the laboratory; and St. Joseph's Hospital (St. Joe's): aspirate collected into an in-house K-EDTA tube, with laboratory personnel in attendance advising on the acceptability of the aspirate, and smears made in the laboratory. Three hematologists from two sites read each of the 129 aspirate slides and a standardized survey was used to determine aspirate acceptability. Unacceptable samples were those rejected by at least two of the three observers.

Results

Results are tabulated in Table 1. St. Joe's had the lowest proportion of unreadable smears at 2 of 40 (5%). MUMC and JHCC had a high proportion of unreadable smears at 23 of 49 (46.9%) and 14 of 40 (35.0%), respectively. Unreadability was largely due to poor aspirate quality (i.e. too dilute, no granules, too few granulocyte precursors) (36 of 39, 92.3%) or to poor smear preparation (i.e. film too thick or cells ruptured) (3 of 39, 7.5%).

Discussion

The most important determinant of sample readability was the quality of the aspirate sample, as 36 of 39 (92.3%) unreadable slides were due to poor aspirate quality. Thus, obtaining a good marrow slide is not dependent on the method of slide preparation but on the expertise of the doctor performing the marrow aspirate. Anticoagulation or non-anticoagulation had no effect on the slide readability as sites that used anticoagulated slides (MUMC) and non-anticoagulated slides (JHCC) both had high levels of unreadability at 46.9% and 35.0%, respectively. Both St. Joe's and MUMC used anticoagulated protocols; however, St. Joe's had better results than MUMC, with only 5.0% of slides unreadable at St. Joe's compared with 46.9% at MUMC. The key difference between these sites is that St. Joe's sends laboratory personnel to the bedside to assess the quality of the aspirate sample, whereas MUMC does not. Thus, the expertise of the technologist in assessing the adequacy of the material at the bedside is also crucial to obtaining a good quality bone marrow sample for slides, biopsy, flow cytometry and cytogenetics. JHCC was the only site with unreadable smears due to poor smear preparation. This makes intuitive sense as these slides must be prepared rapidly in a non-optimal environment prior to sample clotting.

In addition to doctor and technologist expertise, patient type appears to have an impact on the quality of the aspirate obtained. Both JHCC and MUMC have hematology oncology programs and MUMC focuses on pediatric hematology oncology. Clearly, underlying bone marrow disease can make

Table 1. Readable and unreadable slides per site.

Site	N	Readable	Unreadable	Unreadable (Poor Aspirate*)	Unreadable (Poor Smear Prep**)
JHCC	40	26 (65.0%)	14 (35.0%)	11 (27.5%)	3 (7.5%)
MUMC	49	26 (53.1%)	23 (46.9%)	23 (46.9%)	0 (0.0%)
St. Joe's	40	38 (95.0%)	2 (5.0%)	2 (5.0%)	0 (0.0%)
Total	129	90	39	36	3

* Dilute/No Granules/No Granulocyte Precursors / ** Too Thick or Ruptured Cells

the procurement of good bone marrow smears more challenging.

As this was a retrospective study using archived aspirate slides, the study has a number of limitations that must be noted. First, the study focusses only on the method of slide preparation and not on physician, technologist, disease, or the combination of factors. Second, there was no opportunity to assess the ratio of marrow volume to EDTA and its potential effect on cellular morphology. Third, the study was conducted in one multi-sited laboratory program without corroboration of data in a peer multi-sited program. Last, data on the number of aspirates required to obtain each submitted sample was not available.

Notwithstanding the limitations above, a measurable advantage to the use of coagulated aspirate was not demonstrated. Immediate anticoagulation of bone marrow samples with laboratory personnel at the bedside to assess sample quality, followed by slide preparation in the laboratory, provided the best results. Our findings challenge the traditional practice of bedside slide preparation on coagulated aspirate since this method was not associated with improved quality yet is less convenient, requires more laboratory resources in times of increasing constraint and poses increased biohazard exposure risks.

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