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CANCER DIAGNOSES PROFILE OF BONE MARROW SPECIMENS FROM NOVEMBER 2012 TO DECEMBER 2015 IN A TERTIARY ACADEMIC SETTING

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ABSTRACT

This study aimed to examine the number and type of cancer diagnoses made based on bone marrow aspirates (BMA) using morphology and/or histology, over a three year period. All the data collected (2012-2015) was obtained using the Trak-Care laboratory information system (LIS) of the National Health Laboratory Service (NHLS). Data was categorised into four categories including acute leukaemias (ALs), chronic leukaemias (CLs), Hodgkin's lymphoma (HL), Non-Hodgkin's lymphoma (NHL) and a miscellaneous group.

The laboratory test most frequently used to make diagnoses was bone marrow aspiration (BMA) morphology (199), followed by flow cytometry (138), histology (113), fluorescent in situ hybridisation (FISH) (54) and polymerase chain reaction (PCR) (9). In total, the top three conditions diagnosed were acute myeloid leukaemias (AMLs), chronic myeloid leukaemia (CML) and B-cell acute lymphoblastic leukaemia (B-ALL). There was good agreement between the diagnoses made by BMA morphology, BMT histology and flow cytometry.

Results showed that BMA morphology was the most popular diagnostic test used and that this test had excellent agreement with BMT histology and flow cytometry diagnoses. The most frequently diagnosed conditions were the AMLs.

KEYWORDS

Diagnosis, bone marrow aspirate, morphology, histology

INTRODUCTION

The bone marrow is one of the largest organs in the human body and is responsible for haemopoiesis, blood cell formation.[1] The marrow provides circulating blood with blood cells that include: erythrocytes, platelets and leucocytes (white blood cells (WBC)) all of which are derived from pluripotent stem cells.[2,3] Leucocytes include phagocytes and lymphocytes, also derived from pluripotent stem cells.[2,3] In the foetus the site of haemopoiesis in order is: the yolk sac, liver, spleen and bone marrow. In infants haemopoiesis changes to just the bone marrow. In adults haemopoiesis is located at the proximal ends of the humerus and femur as well as in the central skeleton which includes the vertebra, ribs, sternum, skull, sacrum and pelvis.[1,4]

There are two types of marrow in the body, yellow marrow and red marrow. Yellow marrow is inactive marrow composed of 95% fat cells and 5% non-fat cells and makes up half of the total bone marrow in the body. The other half of the bone marrow in the body is red marrow, composed of 40% fat cells and 60% haematopoietic cells and this marrow is haematopoietically active.[2] The yellow marrow can revert into active red marrow if there is a need for increased cell production. Abnormalities in bone marrow function will impair normal activity of the marrow resulting in bone marrow failure.

Both the myeloid leukaemias and lymphocytic leukaemias can be acute or chronic, and this classifies leukaemia into four main types known as: acute myeloid leukaemias (AMLs), acute lymphocytic leukaemias (ALLs), chronic myeloid leukaemia (CML) and chronic lymphocytic leukaemia (CLL). When a patient presents with clinical features and symptoms, related to the function of malignant cells, an initial work up will be carried out. This includes: assessment of medical history, a full blood count (FBC) and a peripheral blood smear for assessment of both the differential WBC count and blood cell morphological evaluation, followed by a bone marrow aspirate (BMA) and a bone marrow trephine (BMT) biopsy. If these tests are not sufficient to establish a definitive diagnosis, immunophenotyping by flow cytometry, cytogenetic analysis by fluorescent in situ hybridisation (FISH), or polymerase chain reaction (PCR) tests can be done.[5]

Bone marrow aspiration (BMA) is a valuable diagnostic tool that can be used for diagnosis where other methods have been unsuccessful. It can also be used to diagnose diseases at an early stage and can therefore allow for early treatment's to be initiated.[6] Both BMA and BMT are complementary, these tests are also useful in follow up tests carried out on patients that are undergoing chemotherapy, bone marrow (stem cell) transplantation or other treatments.[7]

Morphological evaluation of the bone marrow provides information about the composition of the marrow as well as the identification of features that will allow for a successful diagnosis. These features may well be missed, if only a peripheral blood smear were examined.[4] There are however, some pitfalls in using bone marrow morphology and histology to diagnose disease. Inadequate, as for example diluted, samples, can compromise the final diagnoses.[8] An accurate diagnosis can also depend on the experience and knowledge of the pathologist.[9] For adequate bone marrow interpretation the patient's history, clinical findings, peripheral blood picture and other laboratory test results are required.[10] To examine the physical composition of the bone marrow the BMT can be used. BMT biopsy allows for the analysis of the bone marrow stroma as well as the overall bone marrow composition.[11]

The aim of this research project was to examine the number and types of cancer diagnoses, based on bone marrow specimens using morphology and/or histology, with contributing methods, over a three year period. In addition this research project was used to establish a profile of the most commonly diagnosed haematological malignancies, thereby allowing further research into these diseases.

MATERIALS AND METHODS

Data collected over a period of three years (2012-2015) was analysed in the Department of Haematology at the University of Pretoria. All the data was obtained using the Trak-Care laboratory information system (LIS) of the National Health Laboratory Service (NHLS). Case numbers that were successfully diagnosed from November 2012 to December 2015 were included, provided that they were diagnosed using bone marrow specimens. Cases that were reported outside the time period of November 2012 to December 2015 were excluded. If no diagnosis was made this data was also excluded, as well as cases where there was not enough information available. This study was approved by the Research Ethics Committee of the University of Pretoria (109/2016).

RESULTS AND DISCUSSION

A total of 200 patient samples, collected from 2012 to 2015, were analysed in this study. The patients' age and gender were analysed according to year admitted. Table 1 illustrates the baseline demographics for each year.

The specific diagnoses made based on BMA morphology, BMT histology, flow cytometry, FISH and PCR was grouped into four main categories. The four categories were acute leukaemias (ALs), chronic leukaemias (CLs), Hodgkin's lymphoma (HL) and Non-Hodgkin's lymphoma (NHL) and a miscellaneous group. A total of 31 acute leukaemia diagnoses were made using BMA morphology, which was not necessarily a final diagnosis. It should also be noted that two samples had inconclusive diagnoses. The specific diagnoses made using BMT histology shows a reduction in the acute leukaemias diagnosed, T-CLL, B-CLL and non- Hodgkin's lymphoma was diagnosed by histology and not morphology.

Diagnoses using flow cytometry illustrated that there was a reduction in acute leukaemia diagnoses. It also demonstrated the addition of acute biphenotypic leukaemia that could not

Table 1. Baseline demographics of study

YEAR	GENDER	AGE (YEARS)	MEDIAN	AVERAGE
2012	Male = 3 (50%)	7 – 57	28	30.66
	Female = 3 (50%)	3 – 71	46	40
	N = 6	3 – 71	37	35.33
2013	Male = 35 (50%)	1 – 76	20.5	28.37
	Female = 35 (50%)	2 – 83	44	39.09
	N = 70	1 – 83	29.5	33.71
2014	Male = 49 (60%)	0.9 – 80	30	30.14
	Female = 33 (40%)	1 – 84	28	32.39
	N = 82	0.9 – 84	28	31.05
2015	Male = 30 (71%)	1 – 83	33	33.77
	Female = 12 (29%)	1 – 78	35	35.42
	N = 42	1 - 83	33	34.24
TOTAL	Male = 117 (58.5%)	1 – 83	28.5	30.74
	Female = 83 (41.5%)	0.9 – 84	31.5	36.73
	N = 200	0.9 – 84	30	33.58

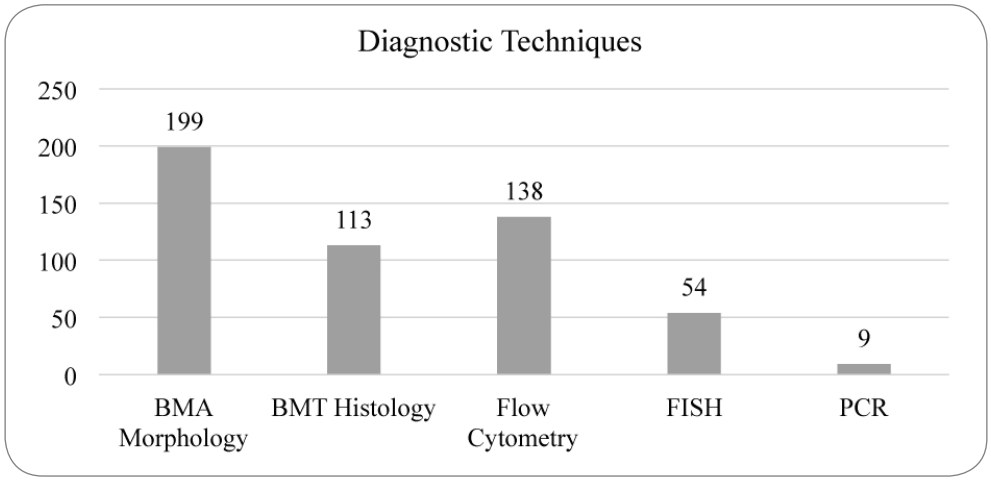

Figure 1. Diagnostic technique frequencies

be diagnosed using just BMA and/or BMT. T-cell chronic lymphocytic leukaemia (T-CLL), Burkitt's lymphoma, carcinoma and neuroblastoma could not be diagnosed using flow cytometry. Two samples were haemodiluted and therefore no definitive diagnosis could be made. There is no specific immunophenotypic signature for CML and the 16 CML diagnoses made were only suggestive of CML.

With regards to FISH and PCR no definitive diagnoses could be made. For FISH certain genes for specific conditions were tested. Results were either positive, confirming diagnosis based on other tests, or negative, not confirming diagnosis based on other tests. Polymerase chain reaction (PCR) was done to test for light chain restriction which then in turn assisted in a successful diagnosis. The correlation between the numbers of times a technique was used and the diagnosis that was finalised based on that technique was assessed. As seen in figure 1 morphology was most frequently used to make a diagnosis, followed by flow cytometry, histology, FISH and lastly PCR.

REVELANCE OF DIAGNOSES

There were a total of 200 samples of which BMA morphology made up 199 (99.5%) of diagnoses. Of those diagnoses, 68.34% were acute leukaemias, 21.61% chronic leukaemias, 1.01% were in the category of Hodgkin's and Non-Hodgkin's lymphoma and 9.05% were in the miscellaneous category. Of the sample size of 200, 113 (56.5%) samples were diagnosed based on histology. The most frequently diagnosed condition were acute leukaemias (55.75%), followed by chronic leukaemias (30.97%). In the category of Hodgkin's and non-Hodgkin's lymphoma, only 1 (0.88%) diagnosis was made. The miscellaneous group had 14 (12.39%) diagnoses.

Flow cytometry was used 138 (69%) times to assist in making a diagnosis. Acute leukaemia was diagnosed a 108 (78.26%) times and chronic leukaemia 24 (17.39%) times. The category of Hodgkin's and Non-Hodgkin's lymphoma only had 1 (0.72%) diagnosis and the miscellaneous category 5 (3.62%) diagnoses. FISH was used in 54 (29.19%) samples, to confirm a diagnosis. Of the 54 times that this technique was used, it correlated with the diagnoses of other techniques 32 (59.26%) times. As with FISH, PCR is used to confirm a diagnosis. Polymerase chain reaction (PCR) was used for 9 (4.59%) samples of 200. Of these 9 samples, the PCR test was positive for 7 (77.78%) samples.

CORRELATION BETWEEN DIAGNOSTIC TECHNIQUES

The correlation between the diagnoses of BMA morphology, BMT histology and flow cytometry was assessed. Cohen's statistic for inter-rate agreement, represented as kappa (κ), was used for this correlation. If $\kappa \leq 0.4$ then there is poor agreement between the tests. If $0.4 < \kappa \leq 0.25$ then there is moderate agreement between the tests. If $\kappa > 0.25$ there is excellent agreement between the tests. The McNemar's test for symmetry was also assessed. This test statistically indicates if the data is symmetrical or asymmetrical, which will represent bias if asymmetrical. If the probability (p-value) for the McNemar's test is > 0.05 there is no symmetry.

There was excellent agreement of 97.32% ($\kappa = 0.9527$) between the BMA morphology test and BMT histology test. The p-value for the correlation was 0.2231 which indicated symmetrical data and no bias towards a certain test.
The agreement between BMA morphology and flow cytometry was 98.54% ($\kappa = 0.9593$) which indicated excellent agreement between these two techniques. This correlation had a p-value of 0.3679 indicating that the data was symmetrical and that there was no bias towards a particular test. The agreement between BMT histology and flow cytometry was 98.61% ($\kappa = 0.9704$) indicating excellent agreement between these two diagnostic tests. The correlation between the tests had a p-value of 0.3173 indicating that the data was symmetrical and that there was no bias towards one of the tests.

Often the BMT and BMA laboratory tests are not sufficient to make a final diagnosis but with the aid of flow cytometry, FISH and PCR a definite final diagnosis is possible. BMA morphology was the most frequently used diagnostic test and histology was only the third most popular laboratory test used. In total, the top three conditions diagnosed were AMLs, CML and B-ALL. The statistical evidence verified that there was excellent agreement between the diagnoses made by BMA morphology, BMT histology and flow cytometry.

These findings support a similar study done by Swart *et al.* at Stellenbosch University in 2007. Swart *et al.* used a sample size

of 124 all of which were referrals to the Fine Aspiration Clinic at the Stellenbosch University. All cases had flow cytometry and cytomorphology that correlated to the bone marrow examination. In 79% of cases an accurate lymphoma diagnosis was made, and they found that flow cytometry could be used successfully to distinguish between benign and malignant lymphoid populations. Their study suggested that fine needle aspiration along with flow cytometry could be used to substantiate a reliable diagnosis. The primary aim of the study was to use these techniques to distinguish between nodal and extra-nodal B-cell populations, but they also found that these techniques could be used to characterise haematological malignancies.[12]

Possible study limitations to this research project included: a relatively small sample size (n=200), a larger sample size would have added credence to the statistical analysis. Another limitation to this study was the lack of data available from other tertiary training facilities.

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