ORIGINAL RESEARCH

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Precision of Histological Bone Marrow Staging in Follicular Lymphoma and Diffuse Large B-cell Lymphoma

Abstract

Introduction: In Non-Hodgkin Lymphoma (NHL), bone marrow histology is the gold standard against which ancillary investigations such as immunophenotyping and gene rearrangement studies are interpreted. There is currently no data on the reproducibility of histological findings. This study was conducted to determine the rates of inter- and intra-observer agreement in histological detection of bone marrow involvement in the two major subtypes of NHL, Diffuse Large B-cell Lymphoma (DLBCL), and Follicular Lymphoma (FL).

Methods: The bone marrow slides of randomly selected DLBCL and FL cases were independently examined by two hematologists using standardized reporting criteria on two occasions at least two weeks apart. Samples included both aspirate and trephine biopsy slides. Weighted kappa statistics were used to examine agreement for the discrete measures.

Results: Weighted kappa analyses showed variable inter-observer agreement in 38 DLBCL cases [aspirate=0.52; trephine= 0.77] and 38 FL cases [aspirate=0.48; trephine=0.77].

Conclusion: Overall, higher agreement rates were noted with trephine biopsies than with aspirates. Except for the high intra-observer agreement on trephine biopsy assessment in FL, there is poor agreement in histological staging of both FL and DLBCL which demonstrates the limitations of histological diagnosis and the futility of interpreting ancillary tests against histology.

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The two commonest subtypes of Non-Hodgkin Lymphoma (NHL) are Diffuse Large B-cell Lymphoma (DLBCL) and Follicular Lymphoma (FL) [1]. DLBCL is an aggressive lymphoma, comprising 30-40% of all cases of B-cell NHL, whereas FL is a low grade NHL, accounting for approximately 30% of B-cell NHL cases [1]. The diagnosis of lymphoma is made on histological assessment of tissue from the primary site of involvement; e.g., lymph node or extranodal tissue [2].

Once the diagnosis is established, staging investigations are performed to determine the extent of spread. This includes radiological assessment using computerised tomography (CT) scans, positron emission tomography, and bone marrow biopsy. Bone marrow (BM) assessment is performed routinely at initial diagnosis in both subtypes of NHL [2] and rates of involvement vary depending on the subtype of NHL. BM involvement is reported in DLBCL in 11-25% of cases [3, 4] and in up to 50% of cases with FL [5, 6].

When assessing the ability of a test to be helpful clinically, it is important to ascertain that the interpretation of results is not a product of conjecture. Precision, as it pertains to agreement between observers (inter-observer agreement), is often reported as a kappa statistic. Kappa is intended to give the reader a quantitative measure of the magnitude of agreement between observers [7].

The histological assessment of the BM is assumed to be reasonably reliable in the hands of experienced morphologists. Surprisingly, there is no objective data or evidence to support this. We therefore performed an inter-observer and intra-observer study using kappa statistic to test the hypothesis that there is a good degree of agreement amongst pathologists over time in the assessment of BM in cases with DLBCL and FL.

Materials and Methods

The reproducibility of histological assessment of BM was studied in randomly-selected cases of DLBCL and FL for whom bone marrow biopsy slides were available at The Canberra Hospital. Approval for the study was obtained from the Australian Capital Territory Health Human Research Ethics Committee. Forty cases of DLBCL were randomly selected using statistical methods by investigator, TC from an established database. A data search was instituted for FL cases and a random selection of 40 cases performed by investigator TC. The BM slides of these cases were retrieved from archives. These included aspirate and trephine biopsy slides.

In our institution, slides performed on aspirate samples include at least two squash preparations, two touch preparations and one touch smear preparation. Trephine biopsies are routinely fixed in formalin and decalcified using nitric acid. Two

Haematoxylin and Eosin stains, one Giemsa stain and a reticulin stain using silver impregnation are prepared. A variable number of sections are cut from the same level – this variation depends on the operator and the biopsy specimen; however, usually for lymphoma cases, as far as possible, three to four sections are cut.

Slides were independently reported by two blinded hematologists (AM and DT) with at least seven years training in evaluating bone marrow aspirate and trephine biopsies. Parameters reported on each BM included cellularity (based on trephine biopsy), lymphomatous involvement on aspirate and trephine biopsy, number of lymphoid aggregates, cellular size of lymphocytes, % of the biopsy involved and presence or absence of fibrosis. Final conclusions of positive, negative or indeterminate for involvement were based on standardized Cheson criteria [8]. Cases positive on aspirate or trephine biopsy were considered positive for involvement.

Both hematologists drew their conclusions without any clinical data and without being aware of the previous reports or reports on ancillary investigations. This was used to assess for inter-observer variability. To assess for intra-observer variability, one hematologist (AM) reported the BM slides on the DLBCL cases a minimum of two weeks apart, and the other (DT) re-reported FL cases. In both cases, the hematologists were not aware of their previous results as cases were re-blinded by the independent investigator TC.

The kappa statistic, which is a measure of the agreement between two observers, was used to assess the agreement amongst the two hematologists. This agreement can range from -1.0 to +1.0; if there is perfect agreement, the value is 1.0, whereas if the observed agreement is what would be expected by chance alone, the value is zero. Kappa statistic is classified as follows: Almost perfect agreement — 0.81 to 0.99, Substantial agreement — 0.61 to 0.80, Moderate agreement — 0.41 to 0.60, Fair agreement — 0.21 to 0.4, Slight agreement — 0.01-0.20, Less than chance agreement <0.01 [7]. Weighted kappa statistic was used for the discrete measures. Computation of results was performed by TC. All statistical analysis was performed by BS and DT using SPSS version 19.0. There were 2 cases each with DLBCL and FL with missing data; these were excluded by the software during statistical analysis.

Results

Adequacy of histological assessment:

Three DLBCL cases did not have aspirate slides; this was confirmed to be due to dry taps. Adequacy of histological assessment for trephine biopsies, as determined by the length of the

Table 1: Adequacy of histological assessment as assessed by trephine length and number of levels examined.

	Follicular lymphoma (n=38)		Diffuse Large B-cell Lymphoma (n=38)	
	Trephine Length (mm)	Number of levels examined	Trephine length (mm)	Number of levels examined
Mean $\pm$ SD	19 $\pm$ 7.91	4 $\pm$ 1.33	18 $\pm$ 7.08	4 $\pm$ 1.47
Maximum	42	8	36	7
Minimum	7	2	8	1
Recommended for adequate diagnosis in lymphoma[25]	16	-	16	4

Table 2a: Inter-observer variability: Number of bone marrows involved with lymphoma at initial assessment

	Diffuse Large B-cell Lymphoma (n=38)			Diffuse Large B-cell Lymphoma (n=38)		
	Investigator 1 (DT)	Investigator 2 (AM)	Discrepant results	Investigator 1 (DT)	Investigator 2 (AM)	Discrepant results
Aspirate	3 (8%)	1 (3%)	2 (5%)	3 (8%)	4 (10.5%)	3 (8%)
Trephine	14 (37%)	14 (37%)	4 (10.5%)	4 (10.5%)	5 (13%)	1 (3%)

Table 2b: Intra-observer variability: Number of bone marrows involved with lymphoma at reassessment

	Follicular Lymphoma (n=38)			Diffuse Large B-cell Lymphoma (n=38)		
	Initial assessment (DT)	Reassessment (DT)	Discrepant results (% total BM reported)	Initial assessment (DT)	Reassessment (DT)	Discrepant results (% total BM reported)
Aspirate	3 (8%)	7 (18%)	4 (10.5%)	2 (5%)	1(3%)	1 (3%)
Trephine	14 (37%)	15 (39.5%)	1 (3%)	6 (16%)	6 (16%)	4 (10.5%)

biopsy and the number of levels examined, was appropriate for Non-Hodgkin Lymphoma [9, 10]. The average length of the trephine biopsies was 18 $\pm$ 7 mm for DLBCL (Range 8-36 mm) cases and 19 $\pm$ 7.9 mm for FL cases (range 7-42 mm) ; the average number of levels examined was 4 $\pm$ 1.5 (range 1-7) for DLBCL and 4 $\pm$ 1.3 (range 2-8) for FL cases [Table 1]. While these specimens were not consistently free of cortical bone, cartilage or connective tissue, these inclusions were not felt to impact significantly on adequacy of histological assessment.

Table 2 shows the rates of involvement in aspirate and trephine samples for DLBCL and FL, as assessed by the two in-

vestigators at initial assessment and reassessment. Over the three assessments, rates of involvement on aspirate and trephine biopsy in DLBCL cases ranged from 3-11% (reflecting 1-4 +ve cases) and 11-16% (reflecting 4-6 +ve cases), respectively. Overall, eight cases were positive for involvement in aspirate and/or trephine samples with six cases positive in trephine biopsies +/- aspirate and an additional two cases +ve in aspirates only. Of these, three were concordant cases with large cell involvement; the remaining four had small cells in the bone marrow.

Much higher rates of involvement were noted in trephine biopsies in FL cases [aspirate: 3-18%, reflecting 1-7 +ve cases, trephine: 37-40%, reflecting 14-15 cases]. Overall, 16 cases were positive for involvement with FL, with 15 cases +ve on trephine +/- aspirate, and one case +ve on aspirate alone. Of the trephine-positive cases, six were also positive in the aspirates. Rates of discrepancies in reporting (BM involved or not involved with lymphoma) between assessments ranged from ~3-11%.

A total of 10 FL cases (five aspirates and five trephines) were identified as having discrepant results on initial assessment between the two investigators and/or on reassessment. Six DLBCL cases (three aspirates and four trephines with one case discrepant on both aspirate and trephine) were identified as having discrepant results either on initial assessment between the two investigators, or on reassessment. All discrepant results on trephine biopsies were evaluated in detail to determine the basis for classifying cases as involved or not involved for lymphoma. All FL cases had a trephine length of at least 16 mm, with two having < two levels examined. Two of the four trephines in DLBCL were <10 mm in length and had < two levels for examination. Reasons for inter-observer and intra-observer discrepancies ranged from differences in the presence or absence of fibrosis, size of aggregates, presence of large cells admixed with small cells and location of aggregates (intertrabecular vs. paratrabecular location). The most discrepant case was reported as exhibiting a 30% involvement with large lymphoma cells in a paratrabecular location in one assessment but as not involved on another assessment. No specific patterns could be identified between FL and DLBCL cases or between inter-observer and intra-observer analysis.

Weighted kappa results showed variable inter-observer agreement in FL: aspirate=0.48 and trephine=0.77. Rates of inter-observer agreement in DLBCL were as follows: aspirate=0.52 and trephine= 0.77. Intra-observer agreement in FL was noted to be: aspirate=0.55 and trephine=0.94. Intra-observer agreement in DLBCL was observed to be as follows: aspirate=0.65 and trephine=0.60.

Intra-observer agreement in FL was noted to be: aspirate=0.55 (91.5% agreement) and trephine=0.94 (97.3% agreement). Intra-observer agreement in DLBCL was observed to be as follows: aspirate=0.65 (97.2% agreement) and trephine=0.60 (89.4% agreement).

Discussion

Most of the literature on reliability studies in lymphoma and leukemia centre on diagnostic samples rather than on staging samples [11-14]. The Non-Hodgkin's Lymphoma Classifica-

tion Project found a diagnostic accuracy of at least 85% for major lymphoma subtypes and diagnostic reproducibility of 85% [15]. The diagnostic accuracy of BM, when it is the primary site of extranodal involvement in NHL, has been reported to be ~85%, with low-grade lymphomas reported to have a diagnostic accuracy of 93% and high grade lymphomas that of ~84% [16].

When assessed for staging, lymphomatous involvement of BM has prognostic significance, as it increases the stage of disease to stage IV. The International prognostic Index (IPI) [17] and the Follicular Lymphoma International Prognostic Index (FLIPI), [18] used in aggressive and low-grade lymphomas, respectively, use BM assessment as one of the tools to determine the stage of disease. Although the use of additional investigations to establish clonality on BM samples has increased, these tests are still often interpreted in the context of histology as the gold standard. Despite the significance of BM assessment in staging lymphoma, there is no literature assessing its reliability in any of the subtypes of NHL. As far as we are aware, this is the first study reporting on the reliability of histological detection of lymphoma in the bone marrow in patients with DLBCL and FL, the two commonest subtypes of NHL. The subjective interpretation of histology in staging bone marrows was apparent in the low levels of agreement across both subtypes of lymphoma; this was least so in the reporting of the trephine biopsies in the patients with FL.

Morphologic detection of lymphoma in the bone marrow can be difficult as compared with non-BM tissue because of several reasons inherent to the lymphoma, the tissue being examined or the pathologist. Patterns of involvement in different subtypes of NHL vary significantly. FL cells characteristically localize loosely to paratrabecular areas with significant fibrosis, making involvement relatively easy to identify. The cytology of cells mirrors that seen in lymph nodes; however, the follicles, which are often seen well on lymph nodes, are rarely seen on BM trephines [1, 9]. In contrast, BM involvement in DLBCL may be quite variable. It may be concordant; i.e., large cells in BM, or discordant; i.e., presence of small cells in BM with only occasional large cells. Discordant involvement has a propensity to be confused relatively easily with benign lymphoid infiltrates. Distinguishing concordant involvement from discordant is important as concordant involvement is associated with a poorer prognosis [3, 19, 20]. Moreover, the malignant cells in DLBCL can occur in the form of diffuse confluent areas, which are relatively easy to identify or scattered large malignant cells, particularly in certain subtypes of DLBCL such as T-cell rich B-cell lymphoma or Anaplastic large B-cell lymphoma,

which may be a lot more difficult to distinguish amongst other bone marrow precursors [1, 21].

Rates of detection of lymphoma on bone marrow aspirates are notoriously low [22, 23]. This may be because of dilution of bone marrow and therefore of possible lymphoma cells by peripheral blood. In cases heavily infiltrated with lymphoma, conversely, the presence of fibrosis may result in a dry tap and a negative bone marrow aspirate [22]. While lymphoid aggregates are relatively easy to identify on trephine biopsy, the diagnosis of lymphoma on the aspirate is based on cytology of individual cells with a consequent increase in variability. Higher rates of agreement were seen in bone marrow trephines as compared with BM aspirates.

Rates of detection of lymphoma in bone marrow trephines can be quite variable because of inadequate sampling. Previously, a number of studies advocated the use of bilateral bone marrow biopsies, [8, 22, 24] although this is no longer felt necessary [2]. Guidelines prescribe a minimum trephine length of 16-20 mm [9, 25]. It is, however, often impracticable to perform a repeat biopsy if this minimum length is not obtained. The number of levels examined can also impact on the rate of detection of lymphoma. For example, in DLBCL, four levels are reported to be a requisite minimum [10]. There is no similar literature available in FL or other subtypes of NHL. In the absence of clear published evidence, four levels were examined for cases with FL. In our cohorts of DLBCL and FL, the mean trephine length and number of levels examined were compliant with recommendations. Although numbers are too small to make meaningful conclusions, it is worth noting that two of the four discordant DLBCL cases had a lower than recommended trephine length.

Many reactive conditions cause benign lymphoid aggregates to form in the bone marrow, which can confound the detection of lymphoma. Histologically, distinguishing these benign lymphoid aggregates from malignant infiltration can be difficult, although features such as size of aggregates and cells, localization, and the presence of increased reticulin are used to distinguish the two [8, 26, 27]. As evidenced in this study, there is a great degree of subjectivity in making such a decision in an individual case – this is likely to be particularly evident in cases with small proportions of lymphocytes. In this study, 10 of the 16 cases with discordant results had <5% involvement with lymphoma cells.

The ICSH guidelines go a long way in establishing standardization in performing and reporting of bone marrow biopsies [25]. However, as these do not adequately cover lymphoma diagnosis specifically, in our study, the Cheson criteria were

used to ensure standardization of reporting of lymphomatous involvement between pathologists over time [8]. BM was reported as positive or negative for involvement, with focus on specific and pre-determined details such as degree, location (paratrabeular *vs.* intertrabeular) and presence of large *vs.* small cells included in the report.

A human factor that contributes to variability in reporting is reporting bias. Clinical details, such as knowledge of the blood count and the clinical/radiological staging results, are often known to the reporting pathologist. This unknowingly leads to a bias in judgment. An even greater contributor to bias is knowledge of results of ancillary investigations. Traditionally, ancillary investigations performed on the BM in staging lymphoma are interpreted against histological diagnosis, which is still used as the gold standard [28, 29]. While a negative result on ancillary investigations such as flow cytometry and immunohistochemistry may convince reporting histopathologists against morphological involvement, literature suggests that this may not necessarily be the case. Lack of concordance between histology and flow cytometry, [30-34] as well as IHC and histology, is well described [21, 35, 36]. Similar irregularities have been shown between histological findings and molecular studies using gene rearrangement studies [37, 38]. While there are no studies comparing the significance of BM and radiological staging, the perceived limited application of the results of BM staging over radiological staging in the management of patients with lymphoma has the potential to introduce a degree of complacency and reduce the reliability of histological diagnosis. These biases were factored into our study by blinding both pathologists to the clinical data as well as to data from ancillary laboratory and radiological investigations.

The obvious limitations of the current study are small cohort of cases and a rather ideal diagnostic situation that may not reflect real-life, where samples may be inadequate and reporting pathologists may not be blinded to clinical data and data from ancillary investigations. This ideal diagnostic situation was, however, necessary for a reliable study of the precision of histological analysis. This study is also important because it explains, to some extent, the widely reported range of BM involvement in studies of FL and DBCFL. It also raises important questions of how best to define BM involvement within prognostic scores such as IPI and FLIPI.

It has been previously suggested that staging in DLBCL can be improved by adding results of ancillary investigations to histological assessment. Of 156 DLBCL cases, immunophenotyping (flow cytometry and immunohistochemistry) upstaged 30 (19.2%) cases to stage IV. A further eight (5.1%) cases were upstaged using molecular studies. A change in IPI was noted in

18 cases (11.5%) on immunophenotyping alone, and 22 (14.1%) cases on immunophenotyping and molecular testing. A revised IPI model, using immunophenotyping with histological assessment, showed better differentiation between IPI categories as compared to baseline IPI using histology alone [4]. The results from the current study help explain the above findings, and further suggest that histological assessment alone is unreliable and is best used in conjunction with ancillary investigations, which, lending themselves to objective data, are likely to be more reliable. Larger multicentre studies are required to determine the impact of using multiple laboratory modalities to detect bone marrow involvement on prognostic indices.

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References

1. Swerdlow S.H. CE, Harris N.L., Jaffe E.S., Plieri S.A., Stein H., Thiele J., Vardiman J.W. (Eds). WHO classification of tumours of haemopoietic and lymphoid tissues IARC: Lyon 2008. 2008.
2. Parker A BB, Devereux S, Gatter K, Jack A, Matutes E, Rooney N, Ross F, Wilkins B, Wotherspoon A, Ramsay A. . Best practice in lymphoma diagnosis and reporting. British Committee for Standards in Haematology, Royal College of Pathologists. 2008.
3. Chung R, Lai R, Wei P, Lee J, Hanson J, Belch AR, et al. Concordant but not discordant bone marrow involvement in diffuse large B-cell lymphoma predicts a poor clinical outcome independent of the International Prognostic Index. *Blood*. 2007 Aug 15;110(4):1278-82.
4. Talaulikar D, Shadbolt B, Dahlstrom JE, McDonald A. Routine use of ancillary investigations in staging diffuse large B-cell lymphoma improves the International Prognostic Index (IPI). *J Hematol Oncol*. 2009;2:49.
5. Lai HS, Tien HF, Hsieh HC, Chen YC, Su IJ, Wang CH, et al. Bone marrow involvement in non-Hodgkin's lymphoma. *Taiwan Yi Xue Hui Za Zhi*. 1989 Feb;88(2):114-21.
6. Arber DA, George TI. Bone marrow biopsy involvement by non-Hodgkin's lymphoma: frequency of lymphoma types, patterns, blood involvement, and discordance with other sites in 450 specimens. *Am J Surg Pathol*. 2005 Dec;29(12):1549-57.
7. Viera AJ, Garrett JM. Understanding interobserver agreement: the kappa statistic. *Fam Med*. 2005 May;37(5):360-3.
8. Cheson BD, Horning SJ, Coiffier B, Shipp MA, Fisher RI, Connors JM, et al. Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas. NCI Sponsored International Working Group. *J Clin Oncol*. 1999 Apr;17(4):1244.
9. Bain BJ. Bone marrow trephine biopsy. *J Clin Pathol*. 2001 Oct;54(10):737-42.
10. Campbell JK, Matthews JP, Seymour JF, Wolf MM, Juneja SK. Optimum trephine length in the assessment of bone marrow involvement in patients with diffuse large cell lymphoma. *Ann Oncol*. 2003 Feb;14(2):273-6.
11. Diebold J, Weisenburger D, MacLennan KA, Muller-Hermelink HK, Nathwani BN, Harris NL, et al. [Reproducibility and prognostic value of histopathological classifications of malignant lymphomas. Prolegomena for the 1st international classification proposed by WHO. Group of the non-Hodgkin's Malignant Lymphoma Classification Project]. *Bull Acad Natl Med*. 1998;182(7):1537-48; discussion 48-9.
12. Chudgar U, Khanduri U. Intraobserver and interobserver reproducibility of the FAB classification in acute leukaemia. *Indian J Pathol Microbiol*. 1992 Jul;35(3):229-36.
13. A clinical evaluation of the International Lymphoma Study Group classification of non-Hodgkin's lymphoma. The Non-Hodgkin's Lymphoma Classification Project. *Blood*. 1997 Jun 1;89(11):3909-18.
14. Hanby AM, Hall PA, Rooney N, Dennis P, James P, Richman P, et al. An inter-observer and intra-observer variability study on the diagnosis of lymph node biopsy specimens. *Eur J Cancer*. 1992;28A(11):1858-62.
15. Fend F, Bock O, Kremer M, Specht K, Quintanilla-Martinez L. Ancillary techniques in bone marrow pathology: molecular diagnostics on bone marrow trephine biopsies. *Virchows Arch*. 2005 Dec;447(6):909-19.
16. Buhr T, Langer F, Schlue J, von Wasielewski R, Lehmann U, Braumann D, et al. Reliability of lymphoma classification in bone marrow trephines. *Br J Haematol*. 2002 Aug;118(2):470-6.
17. Shipp MA, Harrington, D.P., Anderson, J.R., Armitage, J.O., Bonadonna, G., Brittinger, G., Cabanillas, F., Canellos, G.P., Coiffier, B., Connors, J.M., Cowan R.A., Crowther, D., Dahlberg, S., Engelhard, M., Fisher, R.I., Gisselbrecht, C., Horning, S.J., Lepage, E., Lister, T.A., Meerwald, J.H., Montserrat, E., Nissen, N.I., Oken, M.M., Peterson, B.A., Tondini, C., Velasquez, W.A., & Yeap, B.Y. . A predictive model for aggressive non-Hodgkin's lymphoma. The International Non-Hodgkin's Lymphoma Prognostic Factors Project. *N Engl J Med*. 1993 Sep 30;329(14):987-94.
18. Solal-Celigny P, Roy P, Colombat P, White J, Armitage JO, Arranz-Saez R, et al. Follicular lymphoma international prognostic index. *Blood*. 2004 Sep 1;104(5):1258-65.
19. Campbell J, Seymour JF, Matthews J, Wolf M, Stone J, Juneja S. The prognostic impact of bone marrow involvement in patients with diffuse large cell lymphoma varies according to the degree of infiltration and presence of discordant marrow involvement. *Eur J Haematol*. 2006 Jun;76(6):473-80.

20. Conlan MG, Bast M, Armitage JO, Weisenburger DD. Bone marrow involvement by non-Hodgkin's lymphoma: the clinical significance of morphologic discordance between the lymph node and bone marrow. Nebraska Lymphoma Study Group. *J Clin Oncol*. 1990 Jul;8(7):1163-72.
21. Fraga M, Brousset P, Schlaifer D, Payen C, Robert A, Rubie H, et al. Bone marrow involvement in anaplastic large cell lymphoma. Immunohistochemical detection of minimal disease and its prognostic significance. *Am J Clin Pathol*. 1995 Jan;103(1):82-9.
22. Juneja SK, Wolf MM, Cooper IA. Value of bilateral bone marrow biopsy specimens in non-Hodgkin's lymphoma. *J Clin Pathol*. 1990 Aug;43(8):630-2.
23. Sah SP, Matutes E, Wotherspoon AC, Morilla R, Catovsky D. A comparison of flow cytometry, bone marrow biopsy, and bone marrow aspirates in the detection of lymphoid infiltration in B cell disorders. *J Clin Pathol*. 2003 Feb;56(2):129-32.
24. Wang J, Weiss LM, Chang KL, Slovak ML, Gaal K, Forman SJ, et al. Diagnostic utility of bilateral bone marrow examination: significance of morphologic and ancillary technique study in malignancy. *Cancer*. 2002 Mar 1;94(5):1522-31.
25. Lee SH, Erber WN, Porwit A, Tomonaga M, Peterson LC. ICSH guidelines for the standardization of bone marrow specimens and reports. *Int J Lab Hematol*. 2008 Oct;30(5):349-64.
26. Thiele J, Zirbes TK, Kvasnicka HM, Fischer R. Focal lymphoid aggregates (nodules) in bone marrow biopsies: differentiation between benign hyperplasia and malignant lymphoma--a practical guideline. *J Clin Pathol*. 1999 Apr;52(4):294-300.
27. Riley RS, Williams D, Ross M, Zhao S, Chesney A, Clark BD, et al. Bone marrow aspirate and biopsy: a pathologist's perspective. II. interpretation of the bone marrow aspirate and biopsy. *J Clin Lab Anal*. 2009;23(5):259-307.
28. Crotty PL, Smith BR, Tallini G. Morphologic, immunophenotypic, and molecular evaluation of bone marrow involvement in non-Hodgkin's lymphoma. *Diagn Mol Pathol*. 1998 Apr;7(2):90-5.
29. Kang YH, Park CJ, Seo EJ, Huh J, Kim SB, Kang YK, et al. Polymerase chain reaction-based diagnosis of bone marrow involvement in 170 cases of non-Hodgkin lymphoma. *Cancer*. 2002 Jun 15;94(12):3073-82.
30. Talaulikar D SB, Bell J, Khan K, Dahlstrom JE, McDonald A, Pidcock ME, Broomfield A. Clinical role of flow cytometry in redefining bone marrow involvement in Diffuse Large B-cell Lymphoma (DLBCL) – a new perspective. *Histopathology*. 2008;52:340-7.
31. Palacio C, Acebedo G, Navarrete M, Ruiz-Marcellan C, Sanchez C, Blanco A, et al. Flow cytometry in the bone marrow evaluation of follicular and diffuse large B-cell lymphomas. *Haematologica*. 2001 Sep;86(9):934-40.
32. Hanson CA, Kurtin PJ, Katzmman JA, Hoyer JD, Li CY, Hodnefield JM, et al. Immunophenotypic analysis of peripheral blood and bone marrow in the staging of B-cell malignant lymphoma. *Blood*. 1999 Dec 1;94(11):3889-96.
33. Naughton MJ, Hess JL, Zutter MM, Bartlett NL. Bone marrow staging in patients with non-Hodgkin's lymphoma: is flow cytometry a useful test? *Cancer*. 1998 Mar 15;82(6):1154-9.
34. Gomyo H, Shimoyama M, Minagawa K, Yakushijin K, Urahama N, Okamura A, et al. Morphologic, flow cytometric and cytogenetic evaluation of bone marrow involvement in B-cell lymphoma. *Haematologica*. 2003 Dec;88(12):1358-65.
35. Chetty R, Echezarreta G, Comley M, Gatter K. Immunohistochemistry in apparently normal bone marrow trephine specimens from patients with nodal follicular lymphoma. *J Clin Pathol*. 1995 Nov;48(11):1035-8.
36. Talaulikar D DJ, Shadbolt B, Broomfield A, McDonald A. Role of immunohistochemistry in staging diffuse large B-cell lymphoma (DLBCL) *Journal of Histochemistry and Cytochemistry*. 2008;in press.
37. Maes B, Achten R, Demunter A, Peeters B, Verhoef G, De Wolf-Peeters C. Evaluation of B cell lymphoid infiltrates in bone marrow biopsies by morphology, immunohistochemistry, and molecular analysis. *J Clin Pathol*. 2000 Nov;53(11):835-40.
38. Mitterbauer-Hohendanner G, Mannhalter C, Winkler K, Mitterbauer M, Skrabs C, Chott A, et al. Prognostic significance of molecular staging by PCR-amplification of immunoglobulin gene rearrangements in diffuse large B-cell lymphoma (DLBCL). *Leukemia*. 2004 Jun;18(6):1102-7.