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The Role of a Routine Bone Marrow Biopsy in Autoimmune Hemolytic Anemia for the Detection of an Underlying Lymphoproliferative Disorder

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Autoimmune hemolytic anemia (AIHA) occurs at an annual incidence of 1 per 100,000.[1] AIHA can be primary or secondary to conditions such as a lymphoproliferative disorder (LPD), infection, or autoimmunity. The diagnostic approach involves confirming AIHA and screening for secondary causes. Secondary AIHA comprises 50% to 60% of cases, and LPDs account for 15% to 25% of all secondary cases.[2–4] Among the LPDs, AIHA occurs most frequently in chronic lymphocytic leukemia (CLL; 5%–10%), B-cell non-Hodgkin lymphomas (2%–3%), and angioimmunoblastic T-cell lymphoma (AITL; 13%–19%).[4–8] Patients with AIHA secondary to an LPD do not respond as well to corticosteroids and intravenous immunoglobulin alone compared with antilymphoma therapy.[3,9] Therefore, the identification of an LPD is vital to guide therapy. Consequently, imaging with computed tomography (CT) or positron emission tomography and bone marrow (BM) biopsy are frequently performed to screen for an LPD.

Despite this practice, there are limited data regarding the usefulness of a routine BM biopsy to diagnose LPD in newly diagnosed AIHA. A recent international guideline recommends a BM biopsy and flow cytometry in all cold agglutinin disease (CAD) cases before therapy and should be considered in warm and mixed AIHA patients who relapse after steroid therapy.[10] In a French retrospective study, half the patients with newly diagnosed warm AIHA underwent a BM biopsy based on physician preference with the presence of lymphadenopathy, a paraprotein, or hypogammaglobulinemia as the reasons for performing a BM biopsy.[2] Considering the importance of identifying an LPD, many centers, including ours, have performed a routine BM biopsy. We aimed to examine the diagnostic yield of a routine BM biopsy to diagnose an LPD in patients with newly diagnosed AIHA.

We conducted a single-centre retrospective study of all patients undergoing routine BM biopsy to screen for an LPD

with newly diagnosed AIHA between 2000 and 2018 at the Princess Alexandra Hospital, a tertiary referral centre for a large health district. We define BM biopsy as morphological examination of the BM aspirate and trephine and flow cytometry of the aspirate. During this period, our unit policy was to routinely perform a BM biopsy and CT imaging on all patients. Our pathology database was searched to identify all patients fulfilling the following criteria: (1) anemia (≤115 g/L) with a positive direct antiglobulin test (DAT) with IgG and/or C3d; (2) features of hemolysis defined as an absent/reduced haptoglobin and/or elevated lactate dehydrogenase (LDH) and/or elevated unconjugated bilirubin. We classified AIHA based on the DAT result: warm (IgG), C3d (cold), or IgG and C3d (mixed). Patients were excluded if they had a history of an LPD (including CLL) or another acquired or hereditary cause of hemolysis. The pathology database was reviewed for beta-2 microglobulin (β2M), peripheral blood flow cytometry, serum protein electrophoresis, and BM biopsy results. Medical records were reviewed to assess for the presence of B symptoms (fever >38°C, >10% loss of body weight in prior 6 months and night sweats). CT imaging was reviewed to assess for lymphadenopathy or hepatosplenomegaly. Data were collated using Microsoft Excel, and descriptive statistics were performed using SPSS 27.0.1. Comparisons between groups were performed using the Mann-Whitney U test for non-normally distributed continuous variables, Student t test for normally distributed continuous variables, and χ^2 or Fisher exact test as appropriate for categorical variables. Two-sided P of 0.05 was considered statistically significant. This study was approved by the Queensland Metro South Health Human Research Ethics Committee.

A total of 99 patients were identified with anemia, a positive DAT, and biochemical evidence of hemolysis. Twelve patients were excluded due to a known history of an LPD. The remaining 87 patients are the subjects of this study. Baseline characteristics are shown in Table 1 for patients with and without a diagnosis of an LPD. We divided the 87 patients into groups on the basis of the presence of B symptoms, laboratory features (abnormal lymphocytes on the blood film, presence of a paraprotein, positive flow cytometry of the peripheral blood), or imaging features (lymphadenopathy, hepatomegaly, and splenomegaly) suggestive of LPD and whether a diagnosis of LPD was made. Thirty-six patients had no features of an LPD, and no diagnosis of an LPD was made. Thirty-two patients had at least one feature of an LPD, but no diagnosis of an LPD was made; the commonest feature was splenomegaly or low-volume lymphadenopathy, which was attributed to an alternative cause (eg, infection, autoimmune disease).

Table 1.

Baseline Patient Characteristics for All Patients

Baseline Characteristic	Patients Without an LPD (n = 68)	Patients With an LPD (n = 19)	P Value
Median age (y [range])	60 (16–87)	68 (40–81)	0.082
Median hemoglobin (g/L)	84 (40–115)	79 (50–106)	0.34
Median reticulocytes (×10/9 L)	166 (15–772)	118 (6–477)	0.36
DAT result			
IgG	41/68 (60%)	1/19 (5%)	<0.001
C3d	8/68 (12%)	6/19 (32%)	0.037
IgG + C3d	19/68 (28%)	12/19 (63%)	0.0046
Any C3d	27/68 (40%)	18/19 (95%)	<0.001
Median unconjugated bilirubin (µmol/L [range])	37 (7–211)	44 (22–330)	0.146
Raised LDH[a]	60/68 (88%)	18/18 (100%)	0.19
Median LDH (range)	386 (209–2290)	563 (256–1090)	0.059
Reduced/absent haptoglobin	48/68 (73%)	16/18 (88%)	0.18
B symptoms	2/65 (3%)	5/19 (26%)	<0.001
Aberrant/clonal peripheral blood flow cytometry	1/60[b] (2%)	8/12 (66%)	<0.001
Raised β2M	6/30 (20%)	9/17 (53%)	<0.001
Hypogammaglobulinemia[c]	7/67 (10%)	8/19 (42%)	0.0013
Paraprotein	7/67[d] (10%)	9/19 (47%)	<0.001
IgG	4/67 (6%)	2/19 (10%)	
IgM	2/67 (3%)	6/19 (32%)	
IgG + IgM	1/67 (1%)	1/19 (5%)	

[a]Upper limit of normal, 250 U/L.
[b]Aberrant T-cell population deemed not to represent lymphoma.
[c]Defined as gamma globulins <7 g/L on serum protein electrophoresis.
[d]All ≤2 g/L.
β2M = beta-2 microglobulin; DAT = direct antiglobulin test; LDH = lactate dehydrogenase; LPD = lymphoproliferative disorder.

AIHA was defined as warm (n = 42), cold (n = 14), or mixed (n = 31). AIHA was found to be secondary to an LPD in 22% (n = 19) of cases: warm, n = 1; cold, n = 6; mixed, n = 12. Patients with an LPD had a higher incidence of C3d positivity (cold or mixed AIHA), B symptoms, raised β2M, hypogammaglobulinemia and a paraprotein (particularly IgM), and a trend toward increasing age and a higher LDH compared with patients without an LPD. Histological subtypes were low-grade B-cell LPD unspecified (n = 5), AITL (n = 4), monoclonal B-cell lymphocytosis (MBL; n = 4), mantle cell lymphoma (MCL; n = 2), diffuse large B-cell lymphoma (DLBCL; n = 1), CLL (n = 1), lymphoplasmacytic lymphoma (n = 1), and small lymphocytic lymphoma (n = 1). Eighteen were diagnosed at the time of AIHA. One patient was diagnosed with MCL at repeat BM biopsy 1 month later. BM biopsy was diagnostic in 18 of 19 patients with LPD. Of these 18 cases, morphological examination demonstrated involvement in 13, flow cytometry made a diagnosis in 15, and immunoglobulin polymerase chain reaction was used in 1 case. One patient had a diagnosis of DLBCL made on a lymph node biopsy, and the patient's BM biopsy did not demonstrate involvement. Seventeen of the 19 patients had features suggestive of an LPD separate from the BM biopsy: B symptoms, n = 5; paraprotein, n = 9; abnormal lymphocytes on the blood film, n = 4; positive peripheral blood flow cytometry, n = 7; lymphadenopathy, n = 10; hepatomegaly, n = 2; splenomegaly, n = 6. Only one patient was diagnosed with warm AIHA with an LPD (MBL) without any clinical, imaging, or laboratory features of an LPD. This patient did not have peripheral blood flow cytometry performed, which could have made the diagnosis without a BM biopsy.

Similar to prior studies, approximately 20% of AIHA patients had an underlying LPD and increasing age, the presence of a paraprotein, a DAT positive for C3d with or without IgG, and hypogammaglobulinemia were more common in patients with an underlying LPD.[2,3,5,11] The finding that an LPD was significantly higher in patients with cold or mixed AIHA and that only one patient with warm AIHA was diagnosed with LPD supports a recent international guideline that recommends a BM biopsy and flow cytometry should be performed in cold cases before therapy and should be considered in warm and mixed AIHA patients who relapse after steroid therapy.[10] Almost all patients with an LPD had laboratory, clinical, or imaging features suggesting or confirming the diagnosis before BM biopsy. However, a BM biopsy is a low-risk procedure with the potential to reveal important information about the cause of the patient's AIHA. For patients with features suggestive of an LPD, BM biopsy is required to confirm or exclude the diagnosis. Failure to detect an LPD leads to the incorrect diagnosis of primary AIHA. This may lead to increased morbidity and mortality as treatments are different and inferior responses to steroids and survival have been reported in AIHA secondary to an LPD compared with primary AIHA.[2,12] Confirming an LPD is important for funding purposes to access therapy for an LPD (eg, rituximab). BM biopsy may be the only means of confirming an LPD in patients without a suitable biopsy target.

This study has limitations. We classified AIHA based on the DAT result as warm (IgG), cold (C3d), or mixed (IgG and C3d). Although this provides a framework for classifying AIHA, the terms warm, mixed, and cold are not synonymous with IgG, IgG and C3d, and C3d only, respectively, DAT patterns. We acknowledge variations in DAT patterns (ie, warm AIHA may demonstrate complement fixation) and that a diagnosis of CAD requires a cold agglutinin titre in excess of 64.[10,13] Contemporary and expert pathology review may have increased the identification of the entity CAD-associated LPD.[14,15] Another limitation is that this was a retrospective study performed within a single health district. Conversely, a strength of this study was that BM biopsy and CT imaging were routinely performed throughout this study period, as per our unit policy. This enabled the assessment of the incidence of AIHA secondary to an LPD in all patients.

Table 2.

Baseline Characteristics for 19 Patients With Newly Diagnosed AIHA Secondary to an LPD

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Diagnosis	MCL	DLBCL	CLL	MCL	Low-grade B-LPD	Low-grade B-LPD	Low-grade B-LPD	Low-grade B-LPD	LPL	AITL	AITL	AITL	SLL	MBL (non-CLL)	AITL	MBL (CLL)	MBL (CLL)	Low-grade B-LPD	MBL[a] (CLL)
Age (y)	72	53	59	68	76	77	72	81	66	58	80	72	81	40	68	73	61	60	68
DAT result	IgG/C3d	IgG/C3d	IgG/C3d	C3d	IgG/C3d	IgG/C3d	C3d	C3d	IgG/C3d	IgG/C3d	IgG/C3d	IgG/C3d	C3d	IgG/C3d	IgG/C3d	IgG/C3d	C3d	C3d	IgG
Cold agglutinin titre	ND	ND	ND	8192	ND	ND	128	2048	ND	ND	ND	ND	ND	ND	ND	ND	256	–	ND
Diagnosed on BMAT or LN biopsy	BMAT[b]	LN	BMAT	BMAT	BMAT	BMAT	BMAT	BMAT	BMAT	BMAT/LN	BMAT/LN	BMAT/LN	BMAT	BMAT	BMAT/LN	BMAT	BMAT	BMAT	BMAT
BM involvement by morphology	+	–	+	+	–	+	– (IgH PCR pos)	+	+	+	+	+	+	–	+	+	–	+	–
BM involvement by flow cytometry	+	N/D	+	+	+	+	N/A	+	+	+	–	+	+	+	–	+	+	+	+
PB flow cytometry	N/D	N/D	+	+	+	N/D	N/D	N/D	N/D	+	+	+	+	+	–	–	–	–	N/D
Lymphocyte (×10⁹/L)	0.6	0.7	331	4.6	2.9	1.4	0.6	1.5	1.5	1.8	1.5	0.9	4.7	0.5	0.5	1.7	0.43	2.0	0.58
Abnormal lymphocytes on blood film	N	N	Y	Y	N	Y	Y	N	N	N	N	N	N	N	N	N	N	N	N
Hemoglobin (g/L)	66	82	49	57	79	85	76	79	99	81	100	80	50	67	76	75	106	103	72
Platelet (×10⁹/L)	243	344	229	215	333	221	423	350	395	81	156	246	178	80	216	426	115	268	314
Neutrophil (×10⁹/L)	3.9	14	17	4.7	17	3.8	7.8	7.4	1.7	4.6	11.6	2.9	3.1	1.5	2.4	2.4	2.4	3.7	6.35
Paraprotein	IgM/IgG K 2 g/L each	N	N	IgM K 7g/L	N	IgM K 13g/L	N	IgG K T	IgM K 8g/L	IgG K 2g/L	N	N	IgM K 7g/L	N	N	IgM K T	N	IgM L 2g/L	N
Hypogammaglobulinemia	Y	N	Y	Y	N	N	N	Y	Y	N	N	N	Y	N	N	N	Y	N	N
Lymphadenopathy[c]	N	Y	Y	N/A	N	Y	Y	N	Y	Y	Y	Y	Y	N	Y	N	N	N	N
Hepatomegaly[c]	N	N	N	N/A	N	N	N	N	N	Y	Y	N	N	N	N	N	N	N	N
Splenomegaly[c]	Y	N	Y	N/A	N	Y	N	N	N	Y	Y	N	N	Y	Y	N	N	N	N
Elevated β2M	Y	Y	N	Y	Y	Y	N	N	N	Y	Y	Y	N/A	N/A	Y	N	N	N	N
B symptoms	N	N/A	N	N	N	N	N	N	Y	N	Y	N	Y	Y	Y	N	N	N	N

[a] This case is best classified as MBL, however, we acknowledge that peripheral blood flow cytometry is formally required for this MBL diagnosis.

[b] Diagnosed on repeat BMAT 1 month after presentation.

[c] Determined on imaging.

β2M = beta-2 microglobulin; AIHA = autoimmune hemolytic anemia; AITL = angioimmunoblastic T-cell lymphoma; BM = bone marrow; CLL = chronic lymphocytic leukemia; DAT = direct antiglobulin test; DLBCL = diffuse large B-cell lymphoma; LN = lymph node; LPD = lymphoproliferative disorder; MBL = monoclonal B-cell lymphocytosis; MCL = mantle cell lymphocytosis; ND = not detected; N/A = not available; PB = peripheral blood; PCR = polymerase chain reaction; SLL = small lymphocytic lymphoma; Y = yes.

These data suggest the value of routine BM biopsy as part of the initial workup in all AIHA cases is low in the absence of features suggestive of an LPD. The incidence of LPD was higher in patients with C3d-positive (cold or mixed) AIHA. Considering a BM biopsy is a low-risk procedure with the potential to reveal an LPD, which has important therapeutic implications, the value of a routine BM biopsy is higher in patients with C3d positivity. To our knowledge, our data provide the first systematic assessment of the role of routine BM biopsy in AIHA for the detection of LPD. Our data provide an evidence base for the use of BM biopsy in this context and to support guideline recommendations.

DISCLOSURES

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