haematologica
Journal of the Ferrata Storti Foundation

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Received: April 29, 2026.
Accepted: August 19, 2026.

Citation: Ke Xu, Dr KE XU, June Hyun, Robert Baker, Evan Vitsaras, Nicole Japzon, Shiwen Koay, Jahanzaib Khwaja, Jindriska Lindsay, Charalampia Kyriakou, Michael P Lunn, Rajeev Gupta and Shirley D'Sa. The use of MYD88 and IGH polymerase chain reaction in the diagnosis and follow-up of Bing-Neel syndrome. Haematologica. 2026 Aug 27. doi: 10.3324/haematol.2026.301145 [Epub ahead of print]

The use of MYD88 and IGH polymerase chain reaction in the diagnosis and follow-up of Bing-Neel syndrome

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Key Words:

Bing-Neel syndrome

Lymphoplasmacytic lymphoma

MYD88

IGH

Flow cytometry

Author Contributions

KX designed the retrospective audit. KX and JH analysed the data and wrote up the manuscript. All the authors critically revised the final version of the manuscript.

Statement

This work has already been partially reported at the ASH 2025 meeting: https://doi.org/10.1182/blood-2025-5736. 205 patients instead of 211 patients were included in this paper. Six patients were excluded from the original cohort due to the lack of clinical information in our electronic patient records.

Funding statement

The author received no financial support for the research, authorship, and publication of this article.

Conflicts of Interest

J.L. reports membership on an advisory board in, and other support for attending meetings and/or travel from Bristol Myers Squibb (BMS), Pfizer, J&J, BeOne; and honoraria from J&J, Pfizer; and research funding from BMS, J&J and Pfizer.

S.D. reports honoraria from, membership on an entity's board of directors or advisory committees in, and research funding from Janssen; honoraria from and membership on an entity's board of directors or advisory committees in Kite Pharma and Sanofi; membership on an entity's board of directors or advisory committees in Cellectar; and membership on an entity's board of directors or advisory committees in and research funding from BeiGene.

M.P.L. reports ad hoc advisory boards, particularly on trial design, for Roche, AstraZeneca, Sanofi, UCB, Sanofi, Takeda, Polyneuron, and BeiGene (conference expenses and advisory board); unrestricted speaker fees from BeiGene and Grifols for the production of educational materials; and unrestricted conference expenses from BeiGene and CSL Behring. The remaining authors declare no competing financial interests.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

To the Editor,

Bing-Neel syndrome (BNS) is a rare eponymous disease in which the central nervous system (CNS) is invaded by lymphoplasmacytic lymphoma (LPL). Traditionally, the diagnosis was made by rarely-performed brain biopsies or the identification of clonal cytological evidence of LPL in the cerebrospinal fluid (CSF) [1, 2]. Brain or dural biopsies are invasive and rarely performed except in specialist centres. CSF samples often have low cellularity, and LPL cells degrade rapidly, making cytological identification almost impossible, flow cytometry difficult, and diagnosis challenging. The driver mutation in 93-97% of LPL cases is *MYD88*^{L265P} (WHO5) [3, 4]. Immunoglobulin heavy chain (IGH) gene rearrangements are also frequently observed in LPL. With the rapid advancement of molecular diagnostic technologies, polymerase chain reaction (AS-PCR) identification of *MYD88*^{L265P} and IGH allele-specific are widely available in diagnostic laboratories. In this study, we evaluated the use and efficacy of *MYD88*^{L265P} and IGH-PCR and showed the essential role of these tests for diagnosing and following up BNS.

We retrospectively collected data from electronic patient records. We included CSF samples from all patients with a diagnosis of Bing-Neel syndrome treated at University College London Hospital between 2014 and 2024. The diagnosis of BNS was made according to published guidelines [1]. Sample cellularity was assessed by cytospin cytology. Blood-contaminated samples (with presence of red cell(s) on cytology) were excluded from further analysis. Good cellular samples ($\geq$10 cells in 100 µL of CSF sample by cytology) were assessed by flow cytometry (Beckman Coulter Duraclone) performed as described previously [5] (**Supplementary Table 1**), followed by *MYD88*^{L265P} allele-specific PCR (limit of detection [LOD] 0.25%) and IGH-PCR (LOD 1%) if sufficient sample was available (**Supplementary Figure 1**). Acellular and low cellular samples ($\leq$10 cells in 100 µL of CSF sample by cytology) were not assessed by flow cytometry and only by *MYD88*^{L265P} PCR and IGH-PCR. A cell-based DNA extraction method was used. The characteristic lymphoplasmacytic-cell immunophenotype is positive for CD19, CD20, negative for CD5, CD10 and CD23, with surface immunoglobulin restriction. The LOD of flow cytometry is ≥20 events and the limit of quantification (LOQ) is ≥50 events. This study was conducted in accordance with institutional requirements and the Declaration of Helsinki.

205 samples from 50 patients with an eventual diagnosis of BNS were included. Thirty-three samples were the primary presenting sample, and 172 were follow-up samples. Seventeen patients were diagnosed and treated in the other centres and followed up in our centre. Cytology analysis after cytospin was performed on all samples and showed no evidence of high-grade lymphoma, although this methodology is insufficient to fully exclude a histological transformation.

Thirty-three patients were diagnosed with BNS at our centre in this period. One sample was blood-contaminated and excluded from further analysis. Thirty-two CSF samples were suitable for analysis. 27/32 (84%) samples were of good cellularity. Flow cytometry was performed on all 27 samples; 9/27 (33%) showed light-chain-restricted CD5-CD10-CD19+ B

lymphocytes and all 9 confirmed clonality by *MYD88*^{L265P}/IGH-PCR; 13/27 (48%) showed surface Immunoglobulin negative (Ig-) CD5-CD10-CD19+ B lymphocytes (n=13), which was highly suggestive of clonality and all 13 confirmed clonality by *MYD88*^{L265P}/IGH-PCR. One sample was acellular and four were of low cellularity, and therefore were assessed only by *MYD88*/IGH-PCR. 4/5 (80%) confirmed clonality (**Figure 1**). The positive rate of *MYD88*^{L265P}-PCR was 96% (23/24) and IGH-PCR 84% (21/25) in those presenting diagnostic samples. 30/33 (91%) patients' diagnostic CSF samples confirmed clonal B lymphocytes (**Table 1**). Eight patients had molecular-only diagnosis. All 8 had LPL. Their MRI showed leptomeningeal and cauda equina nerve root enhancement (n=1), leptomeningeal and parenchymal enhancement (n=1), cranial nerve enhancement (n=2), brachial and lumbar plexus enhancement (n=1), no abnormalities (n=3). Their persistent symptoms were headache (n=1), sensory deficit (n=3), cranial nerve symptom (n=1), tinnitus with hearing loss (n=1), motor deficit (n=1), seizure (n=1). Other causes including encephalitis, Guillain-Barré syndrome, anti-MAG neuropathy were excluded. The remaining three eventually required leptomeningeal and nerve biopsy to confirm the BNS diagnosis.

One hundred seventy-two samples were follow-up samples. Thirteen of 172 were unsuitable for further tests (blood contamination [n=11], non-viable sample with cell apoptosis and degradation [n=2]). Of the 159 adequate samples, 74 (47%) had good cellularity. Flow cytometry was performed on 54 of 74 good cellularity samples. 8/54 (15%) detected light-chain-restricted B lymphocytes; 3/8 had sufficient samples for *MYD88* ^{L265P}/IGH-PCR, which all confirmed clonality. 18/54 (33%) detected surface Ig- B lymphocytes, highly suggestive of clonal B lymphocytes. 13 of 18 had sufficient sample for *MYD88*^{L265P}/IGH-PCR and in 12 of 13 (92%) clonality was confirmed. 14/54 showed B lymphocytes without light-chain restriction. Nine of these 14 were sent for *MYD88*^{L265P}/IGH-PCR and none confirmed clonality. 11/54 detected B-lymphocytes below LOQ; 10 of 11 were sent for *MYD88*^{L265P}/IGH-PCR and 2/10 (20%) confirmed clonality. 3/54 failed flow with too few events below LOD. Eighty-five of 172 samples were acellular (n=23) or low cellular (n=62), of which 39 were assessed by *MYD88*^{L265P}/IGH-PCR and 13/39 (30%) confirmed clonality (**Figure 2**).

BNS is a diagnostic challenge [6]. The differential diagnosis includes high-grade transformation [7], intravascular lymphoma, CNS angiitis, autoimmune and viral encephalitis as well as fungal, viral, bacterial and acid-fast opportunists and other neurological issues (referral differentials are wide including neurodegenerative diseases, normal pressure hydrocephalus, metabolic encephalopathies, non-convulsive status and more). The diagnosis of BNS requires clinicopathological correlation and confirmation of B-cell clonality through tissue biopsy or CSF examination [1, 6, 8]. Abnormal leptomeningeal or parenchymal involvement visualised by MRI is supportive but not fully diagnostic of BNS, and a normal brain and spine MRI result does not exclude the diagnosis of BNS. Lumbar puncture should be performed after MRI. Cytology assessment for high-grade lymphoma is important. The flow cytometry should be performed on ultra-fresh CSF, as cells viability decreases rapidly *ex vivo*. Thus, sending fresh samples and establishing a rapid process pathway are essential. The use of pre-analytical

sample stabilization, such as Transfix, has been shown to prevent cellular loss and detection of malignant cells in CSF by flow cytometry [9]. Flow cytometry, *MYD88*^{L265P} and IGH-PCR are essential together to confirm clonality. MYD88 positivity alone may not be sufficient for a BNS diagnosis without compatible clinical and radiologic findings [2]. CSF samples are often of inadequate volume and are insufficient to perform both flow cytometry and molecular analyses. Blood contamination can cause false-positive results. Therefore, clinicians should send the last CSF sample for flow cytometry and molecular testing to minimise the risk of blood contamination from traumatic CSF taps. Comparison between PB and CSF flow cytometry and PCR results can help distinguish true CSF disease from PB contamination in selective cases. With good first-time sampling and rapid multiparametric analysis a tissue biopsy is rarely needed having maximised reliable CSF diagnostics.

In this study, we retrospectively analysed the largest cohort of BNS patients undergoing diagnostic and treatment-monitoring tests in a national centre setting over a 10-year period. Diagnostic and follow-up samples were categorised into those that underwent flow cytometry and molecular tests and those that underwent molecular tests only based on sample cellularity. Both diagnostic and follow-up samples demonstrated significant acellular and low cellularity rates (16% vs 53%) (**Table 1**). The main findings were that, even in good cellular samples, molecular testing demonstrated higher sensitivity than flow cytometry in diagnostic and follow-up samples (**Table 1**). *MYD88*^{L265P}-PCR has higher sensitivity than IGH-PCR (96% vs 84% in diagnostic samples). In acellular or low-cellular samples where flow cytometry could not be performed, molecular tests likewise demonstrated excellent pick-up rates in diagnostic (80%) and follow-up samples (30%) (**Table 1**). Furthermore, molecular tests demonstrated greater versatility than flow cytometry in diagnostic samples, detecting clonality in Ig-negative B cells, B cells without light-chain restriction, and samples with too few B cells.

In our cohort, 91% patients' CSF samples at CNS symptom presentation were diagnostic of BNS, much higher rates than are usually quoted. The remaining 9% patients still required traditional tissue biopsy to confirm BNS diagnosis. Monitoring BNS with routine follow-up CSF sample is not recommended by current guidelines due to a lack of clear evidence of benefit [2]. At our centre, patients are monitored with CSF *MYD88*^{L265P}/IGH-PCR every six months both during active treatment and subsequent consolidation or follow up to confirm response and guide treatment. Patients initially positive for *MYD88*^{L265P}/IGH-PCR on CSF, but negative on post-treatment testing (19/25 patients), didn't subsequently relapse from BNS, indicating the value of *MYD88*^{L265P}/IGH-PCR in treatment response monitoring and disease follow-up [10]. *MYD88*^{L265P} digital-PCR can further quantify depth of response [11]; cell-free DNA approach on fresh CSF sample increases diagnostic sensitivity in low cellular cases [12]. These technologies may enable early detection of relapse and guide treatment escalation or de-escalation in the future.

To conclude, our study demonstrates that molecular testing has higher sensitivity for clonal B-cell detection than flow cytometry and is especially useful for those samples with low cellularity and those otherwise unsuitable for flow cytometry. In combination with typical symptoms, cytologic lymphoplasmacytic lymphocyte morphology, *MYD88* ^{L265P} and IGH-PCR can also be used to diagnose BNS. A multidisciplinary team approach is recommended for the diagnosis of this rare disease.

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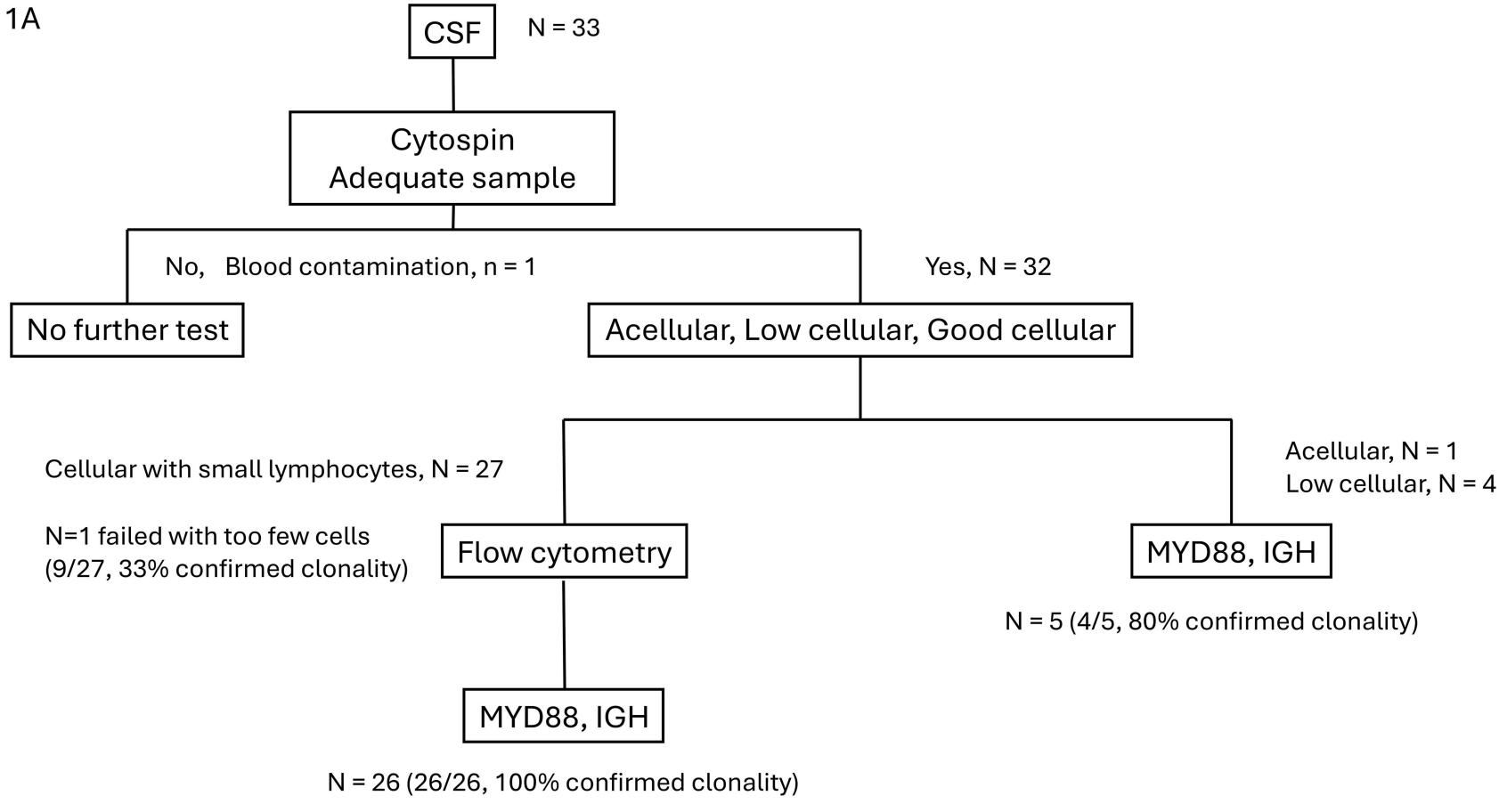
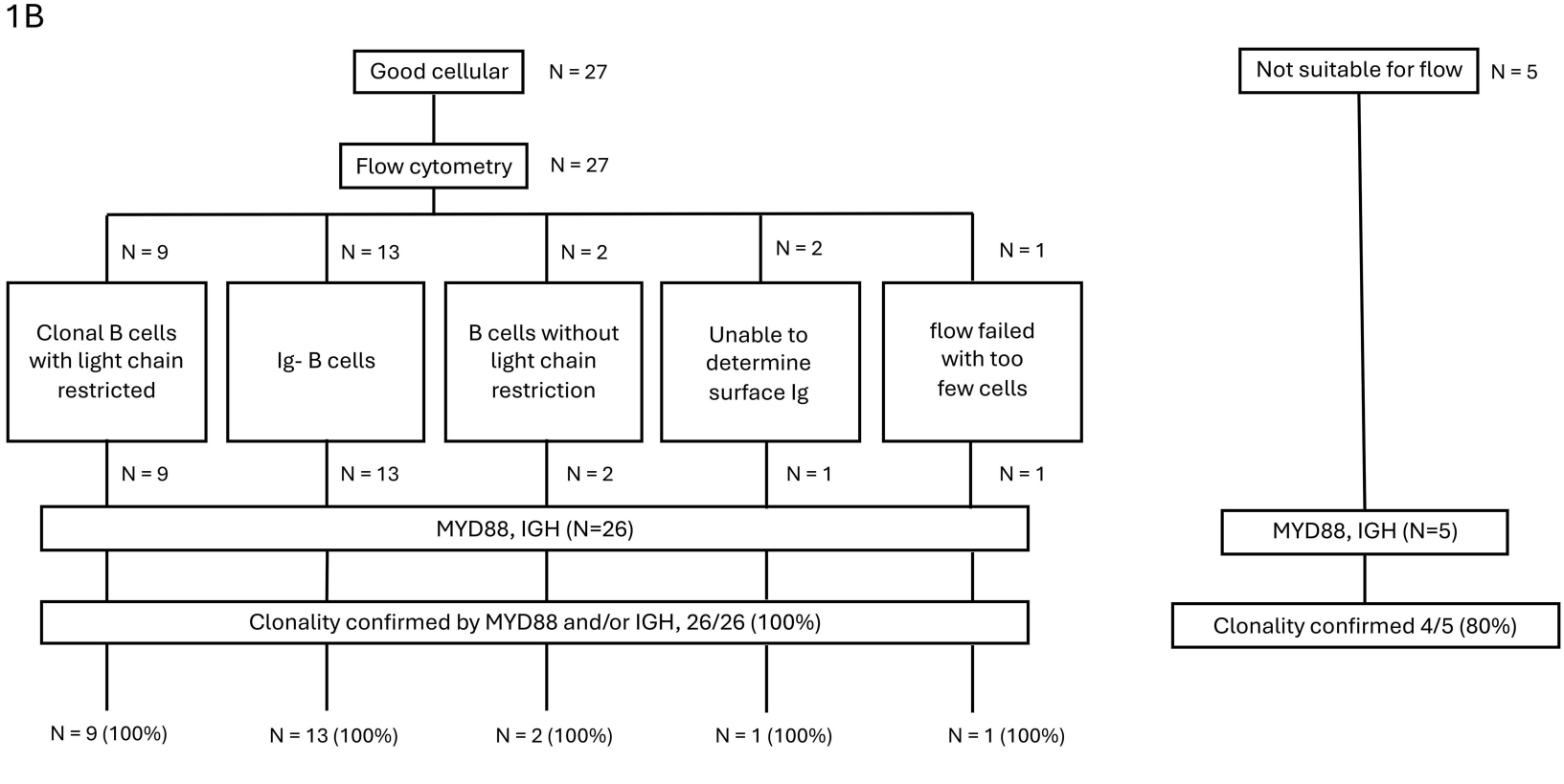
Table 1. CSF test result of patients with Bing-Neel syndrome

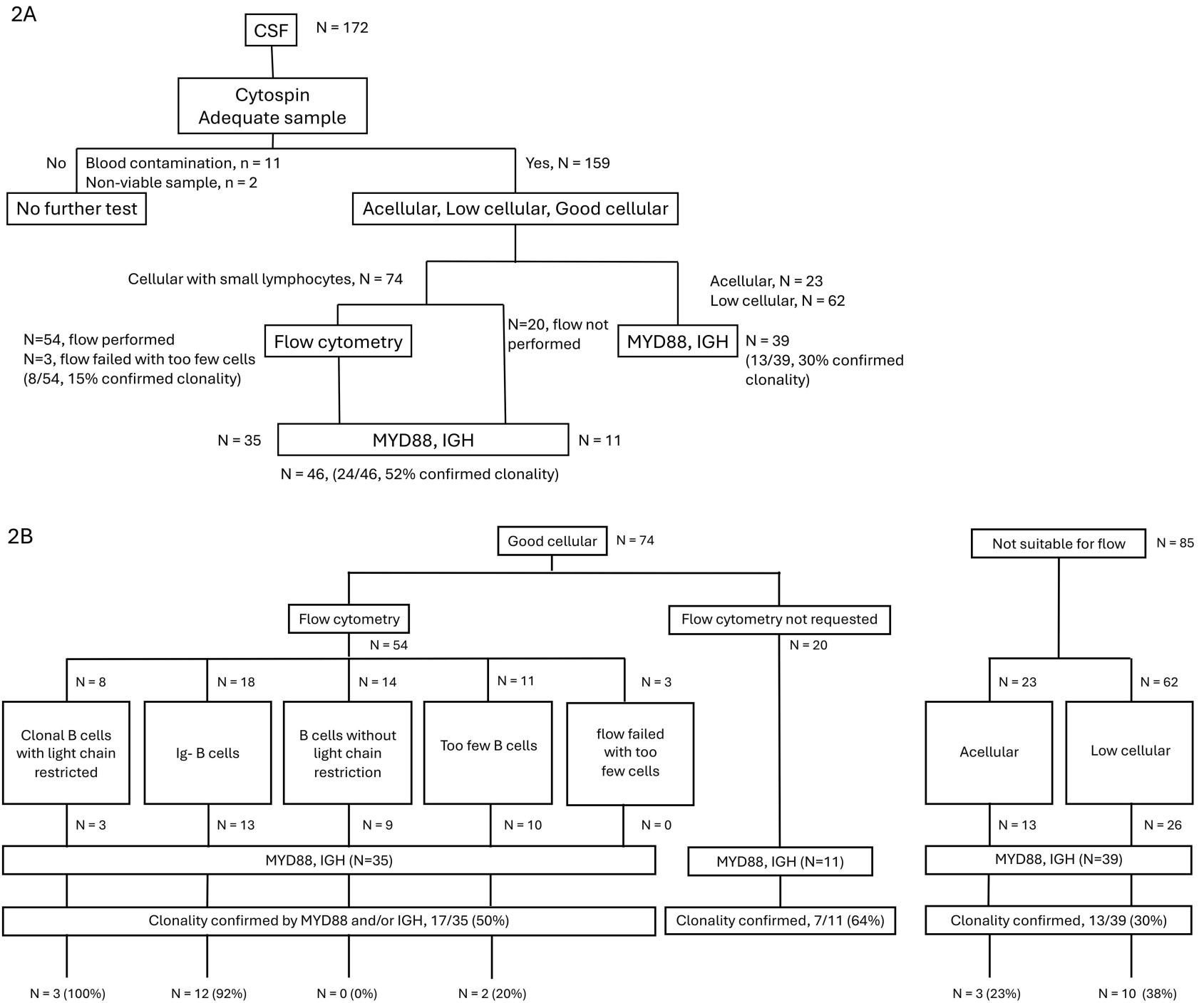
CSF sample (n, %)	**Diagnostic sample (n=33)**	**Follow up sample (n=172)**
Good cellular samples suitable for flow cytometry	27/32 (84%)	74/159 (46.5%)
Flow performed	27	54
Light chain restricted B lymphocytes detected	9/27 (33%)	8/54 (15%)
sIg- B lymphocytes detected	13/27 (48%)	18/54 (33%)
B lymphocytes without light chain restriction	2/27	14/54
Unable to determine sIg due to poor light chain separation	2/27 (15%)	NA
Flow cytometry failed (below LOD)	1/27 (4%)	3/54 (6%)
Below LOQ	NA	11/54 (20%)
MYD88/IGH positive	26/26 (100%)	24/46 (52%)
Acellular or low cellular samples not suitable for flow	5/32 (16%)	85/159 (53%)
MYD88/IGH positive	4/5 (80%)	13/39 (30%)
Samples with confirmed clonality	30/33 (91%)	42/172 (24%)
Clonality confirmed by flow cytometry	9/27 (33%)	8/54 (15%)
Clonality confirmed *MYD88* ^{L265P} PCR	23/24 (96%)	35/76 (46%)
Clonality confirmed IGH PCR	21/25 (84%)	22/67 (33%)

Figure 1. Presenting CSF samples of patients with Bing-Neel syndrome. (A) Algorithm of CSF testing; (B) Flow cytometry, *MYD88* ^{L265P} and *IGH* PCR result.

Figure 2. Follow up CSF samples of patients with Bing-Neel syndrome. (A) Algorithm of CSF testing; (B) Flow cytometry, *MYD88* ^{L265P} and *IGH* PCR result.

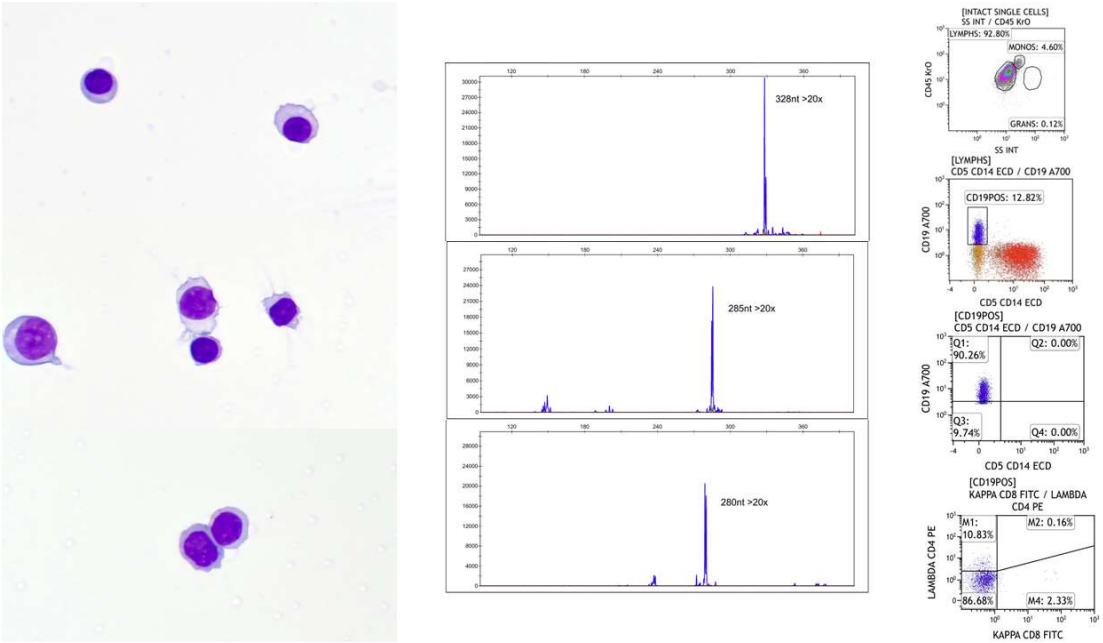

1A
CSF
N = 33
Cytospin
Adequate sample
No, Blood contamination, n = 1
Yes, N = 32
No further test
Acellular, Low cellular, Good cellular
Cellular with small lymphocytes, N = 27
N=1 failed with too few cells
(9/27, 33% confirmed clonality)
Flow cytometry
MYD88, IGH
N = 26 (26/26, 100% confirmed clonality)
Acellular, N = 1
Low cellular, N = 4
MYD88, IGH
N = 5 (4/5, 80% confirmed clonality)


1B
Good cellular
N = 27
Flow cytometry
N = 27
N = 9
N = 13
N = 2
N = 2
N = 1
Clonal B cells with light chain restricted
Ig- B cells
B cells without light chain restriction
Unable to determine surface Ig
flow failed with too few cells
N = 9
N = 13
N = 2
N = 1
N = 1
MYD88, IGH (N=26)
Clonality confirmed by MYD88 and/or IGH, 26/26 (100%)
N = 9 (100%)
N = 13 (100%)
N = 2 (100%)
N = 1 (100%)
N = 1 (100%)
Not suitable for flow
N = 5
MYD88, IGH (N=5)
Clonality confirmed 4/5 (80%)


2A
CSF
N = 172
Cytospin
Adequate sample
No
Blood contamination, n = 11
Non-viable sample, n = 2
No further test
Yes, N = 159
Acellular, Low cellular, Good cellular
Cellular with small lymphocytes, N = 74
Acellular, N = 23
Low cellular, N = 62
N=54, flow performed
N=3, flow failed with too few cells
(8/54, 15% confirmed clonality)
Flow cytometry
N=20, flow not performed
MYD88, IGH
N = 39
(13/39, 30% confirmed clonality)
N = 35
MYD88, IGH
N = 11
N = 46, (24/46, 52% confirmed clonality)
2B
Good cellular
N = 74
Not suitable for flow
N = 85
Flow cytometry
N = 54
Flow cytometry not requested
N = 20
N = 8
N = 18
N = 14
N = 11
N = 3
N = 23
N = 62
Clonal B cells with light chain restricted
Ig- B cells
B cells without light chain restriction
Too few B cells
flow failed with too few cells
Acellular
Low cellular
N = 3
N = 13
N = 9
N = 10
N = 0
N = 13
N = 26
MYD88, IGH (N=35)
MYD88, IGH (N=11)
MYD88, IGH (N=39)
Clonality confirmed by MYD88 and/or IGH, 17/35 (50%)
Clonality confirmed, 7/11 (64%)
Clonality confirmed, 13/39 (30%)
N = 3 (100%)
N = 12 (92%)
N = 0 (0%)
N = 2 (20%)
N = 3 (23%)
N = 10 (38%)

Supplementary Table 1. Monoclonal antibodies and fluorochromes used in the diagnostic flow cytometry panels (Beckman Coulter Duraclone)

Chronic tube (cat no. B93642)	CD3/4/5/8/10/19/20/23/33/34/45/56/Kappa/Lambda
Chronic B tube (cat no. B93612)	CD5/10/19/20/22/25/45/79b/103/FMC7/Kappa/Lambda



Supplementary Figure 1. Good cellular CSF sample from one patient with BNS. (A)Cytospin cytology (x100 objective) showed lymphoplasmacytoid small lymphocytes; (B)Flow cytometry (blue colour population) showed abnormal CD19+CD5-sIg- B lymphocytes; (C)IGH PCR showed clonality.