THE PATTERNS AND DIAGNOSTIC SIGNIFICANCE OF THE LACK OF SURFACE IMMUNOGLOBULIN LIGHT CHAIN ON MATURE B CELLS IN CLINICAL SAMPLES FOR LYMPHOMA WORKUP

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Running Title:

Surface immunoglobulin light chain negative mature B cells

Abstract

Background: Surface immunoglobulin (sIg) light chains are not always detected on mature B cells. This may present as a challenge for clonality determination in clinical flow cytometry.

Methods: To explore the mechanism and diagnostic significance of sIg negative mature B cells, we retrospectively studied 14 cases of sIg negative reactive B-cell lymphocytosis and 89 cases of sIg negative mature B-cell lymphomas. The expression patterns of sIg and cytoplasmic immunoglobulin (cIg) light chains were studied by flow cytometry using both monoclonal and

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/cyto.b.22107

polyclonal antibodies. Results: These 14 cases of sIg negative reactive B-cell lymphocytosis were proven to be polytypic based on cytoplasmic light chain studies. In 89 cases of sIg negative mature B-cell lymphomas, we described 4 distinct patterns of abnormal light chain expression including partial or complete loss of sIg or cIg, suggesting different underlying mechanisms. Conclusions: This study represents the first reported series of body or cystic fluids where reactive B cells do not have detectable sIg, arguing strongly against making a diagnosis of B-cell lymphoma based on lack of sIg in mature B cells. Since the lack of sIg does not always predict clonal/neoplastic mature B-cell proliferation, further cIg evaluation should be performed when sIg expression is not detected in mature B cells. The lack of both sIg and cIg in mature B cells may serve as a reliable surrogate clonality/neoplastic marker.

Key Words:

Mature B cells, lymphoma; flow cytometry; Immunoglobulin light chain

INTRODUCTION

Normal mature B cells express surface immunoglobulin (sIg) kappa or lambda light chains. A population of reactive mature B cells is typically polytypic with admixed kappa+ and lambda+ B cells. In contrast, a population of neoplastic mature B cells is typically monotypic with restricted kappa or lambda light chain expression. In diagnostic workups for patients with lymphadenopathy or lymphocytosis, flow cytometric evaluation of relative proportions of surface kappa+ and lambda+ B-cells (often referred to as a K:L ratio) has been extremely helpful because it can easily differentiate polytypic (reactive) lymphoid proliferation from monotypic (neoplastic) B-cell proliferation (Geary et al., 1993; Reichard et al., 2003). About 3.4% to 12.2% of mature neoplastic B cells have been reported to be negative for immunoglobulin light chain protein (Liendo et al., 1985; de Martini et al., 1988; Batata et al., 1993; Geary et al.,1993; Fukushima et al., 1996; Zardawi et al., 1998; Kaleem et al., 2000; Tomita, et al., 2009; Weinberg et al., 2015; Nakaya et al., 2021). It was suggested that the presence of a significant population of sIg negative B cells can be used as a surrogate marker to diagnose mature B-cell lymphomas (Li et al., 2002). This proposal was challenged because reactive lymphoid hyperplasia may occasionally show expanded sIg negative reactive germinal center B cells (Zhao et al., 2005). There have been very few studies to further explore the mechanism and the diagnostic significance of the absence of sIg light chain proteins in mature B cells. We have encountered a series of cases with sIg light chain negative mature B cells. In these cases, we have studied both sIg and cytoplasmic immunoglobulin (cIg) light chain expression patterns using both monoclonal and polyclonal antibodies, and we found that the loss of light chain expression can be partial or complete at either surface or surface and cytoplasmic levels.

MATERIALS AND METHODS

Selection of samples

To demonstrate the antibody performance in detection of surface and cytoplasmic light chain expression, the listmode data of flow cytometric studies of 30 consecutive cases with pathological diagnosis of reactive lymphoid hyperplasia was retrieved and analyzed.

The cases with samples containing surface light chain negative B-cells were accumulated by authors from 2008 to 2021. These cases were summarized in **Table 1** by disease categories and sample types. The disease categories included reactive lymphocytosis, diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), Burkitt lymphoma/leukemia (BL) and chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL). The specimens where surface light chain negative B-cells detected included body fluid, cystic fluid, bone marrow, lymph node, brain, and thyroid tissue. The study was approved by the Institutional Review Board of Beaumont Health.

Reactive lymphocytosis: 14 cases of reactive lymphocytosis were collected because no surface light chain was detected on B cells. Further evaluation of cytoplasmic light chain expression was performed, and they all showed polytypic expression of cytoplasmic light chain in the B cells. Specimens were collected from pleural fluid (n=9), peritoneal fluid (n=1), pericardial fluid (n=1), cerebrospinal fluid (n=1) and cystic fluid (n=2). Concurrent cytopathology reported predominantly small mature lymphocytes (n=9) or mixed inflammatory cells (n=5). The possible etiology or associated conditions included metastatic cancers (n=6), heart disease (n=3), granulomatous lymphadenitis (n=1), abdominal trauma (n=1), spondylolisthesis pseudo meningocele (n=1) and unknown (n=2).

B-cell lymphoma: 89 cases of sIg light chain negative mature B-cell lymphomas were collected. They included chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL, n=75, specimens including peripheral blood, bone marrow and lymph node), diffuse large B-cell lymphoma (DLBCL, n=8, specimens including body fluid, bone marrow, lymph node, brain, and thyroid tissue), follicular lymphoma (FL, n=4, all from lymph node), Burkitt lymphoma (BL, n=2, both from lymph node).

Flow cytometry immunophenotyping

EDTA or sodium heparin anticoagulated peripheral blood and bone marrow specimens were routinely washed three times with 0.1% BSA/PBS wash buffer. The body fluid samples or cellular preparations mechanically disaggregated from fresh lymph node/soft tissue using forceps and scalpel were routinely washed once and cell suspensions were filtered through a 74 μM nylon mesh to obtain single cells. After appropriate adjustment of cell concentration, aliquots were incubated with a panel of fluorochrome labeled antibodies routinely applied in our laboratories for diagnosis. Red cells were removed with ammonia chloride lysis buffer for blood, bone marrow or other bloody specimens. After initial staining with appropriate surface markers cells were washed and exposed to the reagents in the Fix and Perm Permeabilization Kit (Invitrogen, Waltham, MA) according to the manufacturer's instruction to study for cIg light chains (kappa and lambda). Cells were subsequently incubated with monoclonal or polyclonal antibodies for kappa and lambda light chains. All antibodies were incubated for 15 minutes in dark conditions at room temperature. After washing with PBS, cells were analyzed with a flow cytometer. Different flow cytometers were used depending on the date of specimen received: 5-color flow cytometer FC500 (Beckman Coulter, Indianapolis, IN) for earlier specimens and 10-

color flow cytometer Gallios (Beckman Coulter) for recent specimens. Instrument upgrading and validation were performed in our laboratories according to guideline from College of American Pathologists. The patterns of kappa and lambda light chain expression have been consistent and comparable among these two flow cytometers. The routine immunophenotypic analysis for lymphoma workup used a large panel of antibodies against lymphocyte surface markers: CD45, CD10, CD19, CD20, CD22, CD23, CD38, CD79b, CD2, CD3, CD4, CD5, CD7, CD8, CD16, CD56, and CD57. Light chain reagents included monoclonal antibodies: murine anti-human kappa-FITC (TB28-2, BD Biosciences, San Jose, CA), anti-human kappa-APC (TB28-2, BD Biosciences, San Jose, CA), and anti-human lambda-PE (1-155-2, BD Biosciences, San Jose, CA) and polyclonal antibodies: rabbit anti-human lambda-FITC, and anti-human kappa-PE (Dako, Carpinteria, CA).

Data analysis

The original list mode data of these cases were re-analyzed using CXP or Kaluza analysis software (Beckman-Coulter). B cells were identified based on B-cell markers: CD19 and CD20. Surface light chain expression patterns were analyzed on a kappa versus lambda dot plot. B cells were considered kappa or lambda negative if kappa or lambda staining intensity on B cells was the same as that on the internal negative control populations (T cells stained with light chain antibodies and B cells stained with antibodies against T/NK-cell markers). Cases with appreciable but dim expression of surface light chains were not included in this study.

RESULTS

Surface light chain positive mature B cells

Light chain expression in normal mature B cells have been well described in the literature. For comparative purposes, we re-examined mature B cells of 30 specimens with reactive lymphadenopathy based on pathology diagnosis. Both surface and cytoplasmic light chain expression patterns were reviewed. The mean ratio of surface K+ and surface L+ B cells was 1.40 with a range of 1.05 to 2.15. The mean ratio of cytoplasmic K+ and cytoplasmic L+ B cells was 1.45 with a range of 1.10 to 2.14. K+ B cells generally outnumbered L+ B cells. The patterns of surface and cytoplasmic light chain expression of mature polytypic B cells were very similar. There was no significant difference statistically between surface and cytoplasmic studies (t-test, p=0.46). No significant light chain negative mature B cells were detected in these samples.

Surface light chain negative mature polytypic B cells

Surface light chains were not detected by monoclonal or polyclonal antibodies in mature B cells of body fluids and cystic fluids collected from 14 patients with no clinical and pathological evidence of lymphoma/leukemia. When further evaluated for cytoplasmic light chain expression, these B cells showed typical patterns of polytypic cytoplasmic light chain expression with either monoclonal antibodies or polyclonal antibodies**.** Shown in **Figure 1** is an example of pleural fluid sample with lymphocytic cellular infiltrate of admixed T cells and B cells. CD20 positive B cells showed no detected sIg using monoclonal antibodies. Their cytoplasmic light chain expression appeared normal. The mean cytoplasmic K:L ratio was 1.51 with a range of 1.23 to 1.83. These ratios were not statistically significant from that observed in mature B cells in lymph nodes with reactive hyperplasia (t-test, p= 0.45).

Surface light chain negative mature neoplastic B cells

A total of 89 cases of surface light chain negative mature B-cell lymphomas were collected for this retrospective study. These cases included CLL/SLL (75/89, 84%), DLBCL (8/89, 9%), FL (4/89, 4%) and BL (2/89, 2%). This set of cases was chosen because sIg expression was not detected by flow cytometry using monoclonal antibodies against kappa and lambda. These cases were further evaluated using polyclonal antibodies to study for surface light chain expression, and in certain cases, cytoplasmic light chain expression using monoclonal antibodies, polyclonal antibodies, or both. Four distinct patterns of abnormal light chain expression were observed among these 88 cases of sIg negative mature B-cell lymphomas as summarized in **Table 2**. The first pattern was consistent with partial loss of surface light chain. It was based on the finding that surface light chain expression was not detected by monoclonal antibodies but was detected by polyclonal antibodies. This group was comprised of 1 case of SLL/CLL and 1 case of DLBCL. **Figure 2** shows a lymph node involved by a CLL/SLL. There was no difference in stain intensities on CLL/SLL cells between the monoclonal antibody set against light chains (**Figure 2 A**) and the unrelated monoclonal antibody set against T/NK-cell markers (**Figure 2 B**). The staining intensity of CLL/SLL cells with monoclonal antibodies against kappa and lambda (**Figure 2 A**) was essentially the same when comparing it with the background staining of normal T-cells with the same antibody set (**Figure 2 C**). Restricted light chain expression was apparent when CLL cells were stained with polyclonal antibodies (**Figure 2 D**). For comparison, a small number of residual normal B cells showed normal light chain expression with both monoclonal and polyclonal antibodies. The second pattern was consistent with complete loss of sIg light chain antigen but normal expression of cytoplasmic light chain. Here we observed that surface light chains were not detected with polyclonal antibodies, but cytoplasmic light chains were detected with both monoclonal and polyclonal antibodies. This group was comprised of 68

cases CLL/SLL and 3 cases of DLBCL. An example of this pattern is shown in **Figures 3 A** and **3 B**, representing a lymph node involved by a SLL/CLL. The third pattern was consistent with complete loss of surface light chains and partial loss of cytoplasmic light chains. In this pattern, sIg light chains were not detected in neoplastic B cells with the use of either monoclonal or polyclonal antibodies, while cIg light chains were detectable with polyclonal antibodies but not monoclonal antibodies. This group comprised of 5 cases of CLL/SLL, 1 case of DLBCL, 1 case of FL and 1 case of BL. A representative case with a lymph node involved by a FL is shown in **Figures 3 C** and **3 D.** The fourth pattern was characterized by complete loss of both sIg and cIg light chains. In this pattern, neither surface nor cytoplasmic light chains were detected with monoclonal or polyclonal antibodies. The eight cases identified in this group included 3 cases of DLBCL, 3 cases of FL, 1 case of BL and 1 case of CLL/SLL. **Figure 4** is a lymph node sample involved by a FL. In this example, neoplastic B cells (red) had no detectable cytoplasmic light chain expression with either monoclonal antibodies (**Figure 4 A**) or polyclonal antibodies (**Figure 4 B)**. These neoplastic B cells were identified as germinal center B cells (CD20+, CD10+, red, **Figure 4 C**). In contrast, B cells showed a polytypic pattern of cytoplasmic light chains were identified as non-germinal center B cells (CD20+, CD10-, pink, **Figure 4 C**).

DISCUSSION

Mature B cells are expected to express sIg kappa or lambda light chains. They are considered neoplastic if a significant large mature B-cell population shows sIg light chain expression restricted to kappa or lambda. However, it presents a challenge for diagnostic interpretation when sIg light chains are not detected in a mature B-cell population in clinical flow cytometry practice. Careful correlation with pathology evaluation and additional ancillary diagnostic study is often required for a definitive diagnosis.

We have encountered a variety of clinical samples with surface light chain negative mature B cells detected by flow cytometry over last decade. Some cases were evaluated surface and cytoplasmic light chain expression using monoclonal antibodies and polyclonal antibodies. Among these cases, we observed 4 distinct patterns of abnormal light chain expression in mature neoplastic B cells. We believe these four patterns suggest different biological mechanisms that lead to abnormal light chain expression: 1. abnormal sIg light chain proteins with antigenic epitope loss; 2. failure to transport cIg light chain proteins to the cell surface membrane; 3. abnormal cIg light chain proteins with antigenic epitope loss; 4. complete lack of cIg light chain proteins. When lymphoma cells had abnormal sIg light chain proteins with antigenic epitope loss, B-cell clonality could not be easily determined if only monoclonal anti-light chain antibodies were used. Therefore, concurrent or add-on polyclonal antibody reagents for evaluation of surface light chain expression may provide a definitive answer for clonality since a polyclonal antibody can recognize multiple antigenic epitopes of light chain proteins. If surface light chains are not detected with polyclonal antibody reagents, cytoplasmic light chain study is quite useful to further assess the light chain expression patterns. Our observation is consistent with published literature indicating that polyclonal antibodies are more likely to detect light chain expression than monoclonal antibodies (Horna et al., 2011; van Velzen et al., 2014).

In this study, we were unable to provide the prevalence of absence of sIg from B-cell surface in reactive and tumor samples because these sIg negative cases reported here were sporadically collected over a decade. When we decided to perform this retrospective study, we found it was extremely difficult to retrieve all sIg negative cases. Evaluation of cytoplasmic light chain

expression patterns was not always performed with both monoclonal and polyclonal antibodies. Documentation of their light chain expression patterns has not been consistent among our pathologists who signed out these cases. However, our cases showed a variety of light chain abnormalities manifesting in different expression patterns at both surface and cytoplasmic levels. This may help pathologists to establish appropriate algorithmic approach to handle surface light chain negative cases. It is of great benefit to document these unique light chain expression patterns in detail in flow cytometry reports to guide subsequent diagnostic workup in monitoring therapy response or disease progression.

Both polyclonal and monoclonal antibodies against kappa and lambda have been widely used in clinical flow cytometry. Technically they have their own merits. Polyclonal antibodies have higher affinity and sensitivity while monoclonal antibodies are more specific and exhibit lower background staining. There has been no consensus on which is preferable (O'Donahue et al., 2018). If these 4 patterns of abnormal light chain expression in mature B-cell lymphoma are taken into additional consideration, it is preferable to carry both monoclonal and polyclonal anti-light chain antibodies in clinical flow cytometry laboratory, allowing adequate evaluation of B-cell clonality and light chain abnormality. In our laboratory, we currently carry both monoclonal and polyclonal antibodies against kappa or lambda light chains. For bone marrow and peripheral blood samples, we have routinely evaluated surface light chain expression with two sets of light chain antibodies: monoclonal anti-kappa-FITC/monoclonal anti-lambda-PE; and polyclonal anti-lambda FITC/polyclonal anti-kappa-PE. For lymph node and soft tissue samples, we have routinely evaluated surface light chain expression with monoclonal antibodies and cytoplasmic light chain expression with polyclonal antibodies. If the cell count was low, we would typically

use polyclonal anti-lambda FITC/polyclonal anti-kappa-PE for light chain studies. Based on our experience, evaluation of cIg light chain expression with polyclonal antibodies is the most sensitive in determining light chain restriction. In practice, this can be done initially with a routine antibody panel or with an add-on antibody combination. Monoclonal antibodies typically produce better signal to noise ratios and more consistent results with much less batch-to-batch variation. However, a comparison of light chain staining patterns between two sets of anti-light chain reagents (monoclonal and polyclonal) or between two different staining protocols (surface and cytoplasmic) will reduce ambiguous results and subsequently reduce unnecessary immunohistochemistry stains, cytogenetic or molecular studies.

Our findings argue against using surface light chain negativity as a clonality/neoplastic marker for mature B cells, at least for body or cystic fluid samples. 14 patients with reactive lymphocytosis reported in our study had no history or concurrent B-cell non-Hodgkin lymphoma. The lymphocytosis appeared chronic and reactive with or without significant admixed neutrophils or monocytes. Immunophenotypically these B cells were positive for CD19, CD20 and CD45, but negative for CD10. Dim and partial expression of CD5 was observed in 4/14 cases. The overall immunological or cytological features were consistent with small mature lymphocytes. We could not explain why and how these reactive mature B cells became surface light chain negative. Are these sIg light chain negative B cells actually immature plasma cells (lymphocytes in differentiation toward plasma cells/plasma blasts)? Unfortunately, the CD138 expression was not evaluated with these samples. The CD20 staining pattern of these B cells in these fluid samples was very similar to that seen in normal B cells. CD38 expression was evaluated in two cases, greater than 80% of B cells were positive for CD38. The

relationship between CD20 and CD38 did not suggest a progressive maturation to plasma cells / plasmablasts. Of interest, surface light chain negative, polytypic mature B cells have not been encountered in the blood, bone marrow and lymph node specimens, suggesting this may be unique in these body and cystic fluid samples. In our laboratory, blood and bone marrow samples are routinely washed three times while body fluid and cystic fluid samples are routinely washed once before they are stained for sIg light chains. We could not completely rule out the possibility of insufficient washing off immunoglobulins in these samples which happen to have unusual high-level immunoglobulins. The residual immunoglobins left in the cell samples, if high enough, may completely absorb light chain antibodies before they bind to sIg light chains. Further studies are necessary to explore the possible causes. Protein electrophoreses can be applied to assess the concentration of immunoglobulins and other proteins in the body fluids. In addition, different washing protocols will be tested on their impact on surface light chain staining when such samples are encountered.

Partial and complete lack of cIg light chain has been reported in mature B-cell lymphoma (Bardales et al., 1983; Brozic et al., 2015). Whether this can be used to diagnose lymphoma may require further validation. Extensive PubMed literature search has not identified any report of reactive lymphoid hyperplasia or lymphocytosis with complete cIg light chain loss. Therefore, the lack of both sIg and cIg light chain in mature B cells may serve as a surrogate clonality/neoplastic marker in diagnostic workup. Of course, lack of sIg or cIg light chain should not be interpretated in isolation. In the context of abnormal antigen expression, abnormal light scatter and atypical cytomorphology, the lack of detectable sIg or cIg light chain in mature B cells is in favor of clonal neoplastic B-cell proliferation. When in doubt, it is better

to report the flow cytometric finding as atypical mature B cells detected with no sIg or cIg light chain expression. Further correlation with pathological evaluation and clinical correlation is recommended before making a definitive diagnosis.

The sIg negativity observed in neoplastic B cells in our cases is unlikely due to technical issues. Variable numbers of residual normal B cells are frequently seen in the background with appropriate staining using light chain antibodies (**Figure 2**). To rule out the possibility of false negative staining due to non-bright fluorochrome of light chain antibodies, we compared the staining pattern and intensity between monoclonal anti-kappa-FITC with monoclonal anti-kappa-APC in some cases (data not shown). Since the background staining with different fluorochromes may not be the same. When light chain staining presents ambiguously, it can be interpreted as either dimly positive or negative. Before we decided the light chain staining as dimly positive or negative, we compared the staining patterns between the light chain antibodies and other unrelated antibodies labeled with same fluorochromes. It is possible that very dimly positive light chain staining could be potentially interpreted as negative and vice versa. Cases with appreciable dim expression of sIg light chains with monoclonal antibodies were excluded in this study. The exact biochemical mechanism of absence of sIg light chains needs further study. The most common pattern of light chain abnormality is sIg-/cIg+ in CLL/SLL which represented the largest cohort in our cases. Our findings are consistent with a previous report (Lewis et al., 2005). They were able to demonstrate light chain restriction in most CLL/SLL cases (90%) if cytoplasmic Ig light chain expression was evaluated. Our preliminary studies showed these cases were scattered among different prognostic groups (data not shown) within CLL/SLL cases. The impact on the biological behaviors and clinical outcomes remains to be further studied.

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TABLES

Table 1. Summary of cases with sIg negative B cells

Diagnosis	Case Number (n=103)	Specimens
RL	14	body fluid, cystic fluid
CLL/SLL	75	blood, lymph node, bone marrow
FL	4	lymph node
DLBCL	8	lymph node, brain, thyroid
BL	2	lymph node

Note: RL, reactive lymphocytosis; CLL/SLL, chronic lymphocytic leukemia/small lymphocytic lymphoma; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; BL, Burkitt lymphoma.

Table 2. Patterns of surface light chain negative B cells

	Reactive (n=14)	CLL/SLL (n=75)	DLBCL (n=8)	FL (n=4)	BL (n=2)	Total (n=103)
Pattern 1 sIg m-/sIg p+		1	1			2
Pattern 2 sIg m-/sIg p-/ cIg m+/cIg p+	14	68	3			85
Pattern 3 sIg m-/sIg p-/ cIg m-/cIg p+		5	1	1	1	8
Pattern 4 sIg m-/sIg p-/ cIg m-/cIg p-		1	3	3	1	8

Note: Numerical values represent number of cases for each diagnosis. Pattern 1, partial loss of surface immunoglobulin (sIg): sIg not detected by monoclonal antibody but detected by polyclonal antibody (sIg m-/sIg p+); Pattern 2, complete loss of sIg but normal expression of cIg (sIg m-/sIg p-/ cIg m+/cIg p+); Pattern 3, partial loss of cytoplasmic immunoglobulin (cIg): cIg detected by polyclonal antibody only (sIg m-/sIg p-/cIg m-/cIg p+); Pattern 4, complete loss of cIg: cIg not detected by neither monoclonal antibody nor by polyclonal antibody (sIg m-/sIg p-/cIg m-/cIg p-). CLL/SLL, chronic lymphocytic leukemia/small lymphocytic lymphoma; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; BL, Burkitt lymphoma.

FIGURE LEGENDS

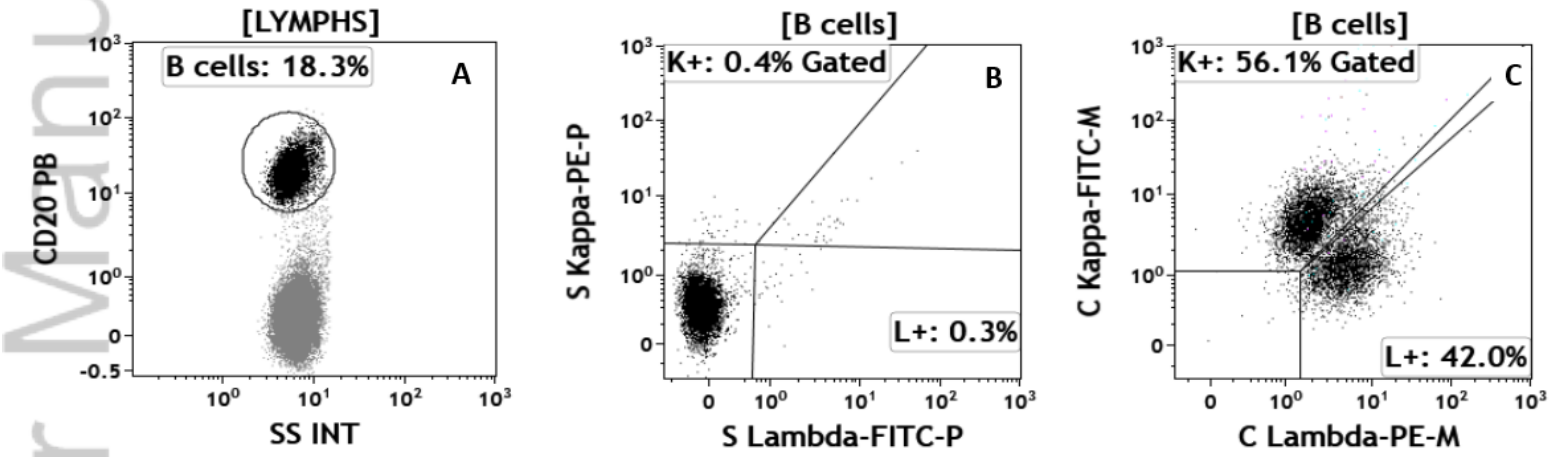
Figure 1. Mature polyclonal B cells without detectable surface light chains. This is a representative example of a body fluid sample. B cells are CD20+ (colored black, plot A). Surface light chain expression is not detected in B cells (plot B) with polyclonal antibodies. Polytypic cytoplasmic light chain expression is detected with monoclonal antibodies (plot C).

Figure 2. Partial loss of surface light chain antigen only. Displayed is an example of CLL/SLL. Plot A shows staining intensity of neoplastic B cells (colored black) and polytypic B cells (highlighted gray) with monoclonal antibodies against kappa and lambda light chains. Plot B shows background staining of B cells with monoclonal antibodies against CD8 and CD57. Plot C

shows background staining of T-cells with monoclonal antibodies against kappa and lambda light chains. Plot D shows detectable surface light chains on neoplastic B cells and polytypic B-cells with polyclonal antibodies.

Figure 3. Expression of cytoplasmic light chains in neoplastic B cells with complete loss of surface light chain antigen. This figure illustrated two different patterns in cytoplastic light chain expression: intact (Plots A and B, an example of CLL/SLL) and partially lost (Plots C and D, an example of FL). In the example of CLL/SLL, cytoplasmic light chain expression is detected in neoplastic B cells using either monoclonal (plot A) or polyclonal (plot B) antibodies. In the example of FL, cytoplasmic light chain expression is not detected in neoplastic B-cells (colored black) using either monoclonal (plot C) or polyclonal (plot D) antibodies. In contrast, normal light chain expression is seen some residual polytypic B cells (colored gray).

Figure 4. Complete loss of surface and cytoplasmic light chain antigen. Displayed is an example of FL. The stains with monoclonal antibodies (plot A) and polyclonal antibodies (plot B) show no cytoplasmic light chain expression in neoplastic B cells (black). In contrast, polytypic cytoplasmic light chain expression of residual normal B cells (gray) is detected as expected with either monoclonal or polyclonal antibodies. Plot C shows differential expression of CD20 and CD10. Neoplastic B cells are CD10+CD20+ brighter. Residual normal B cells are CD10-CD20+ dimmer.



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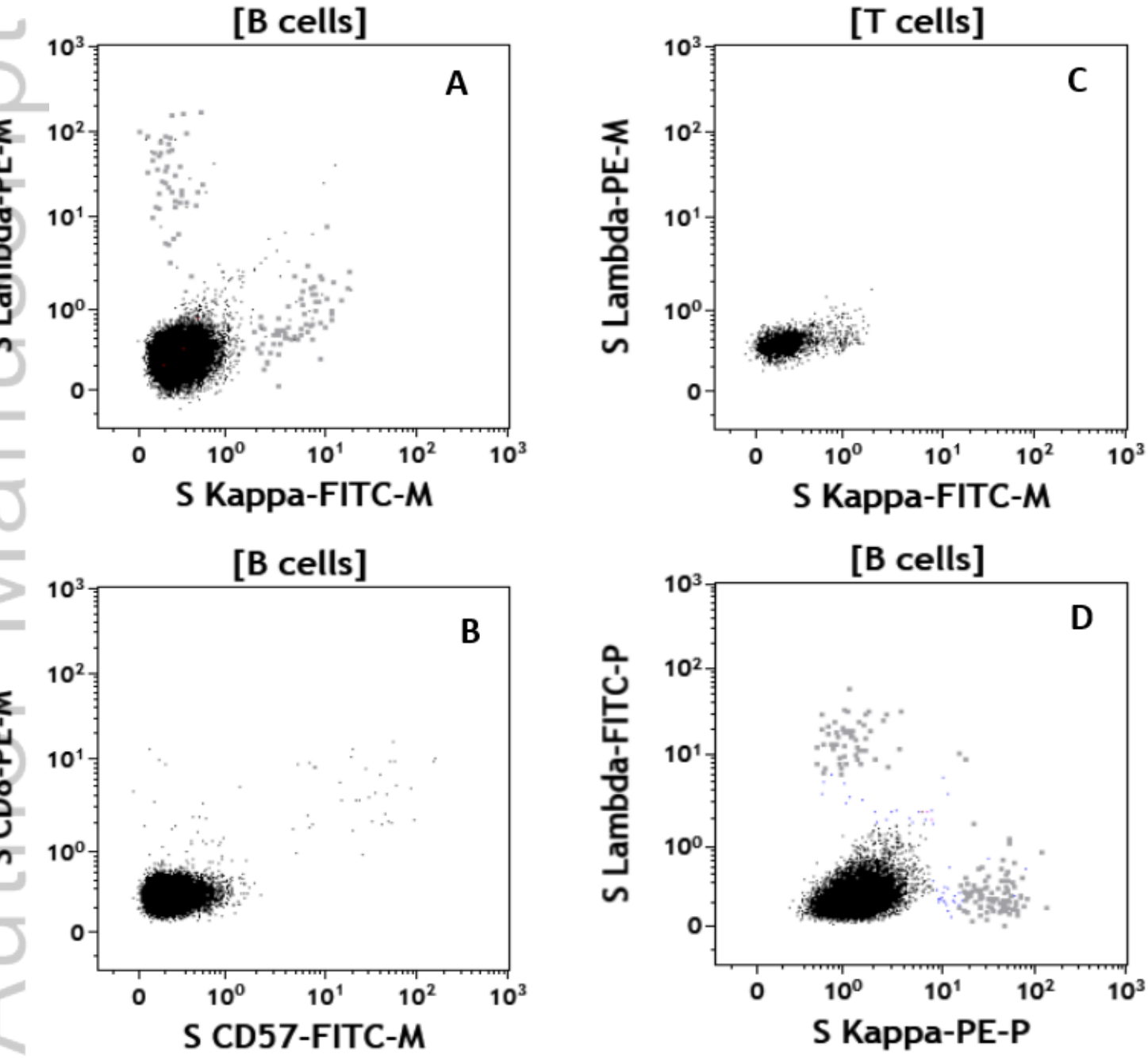

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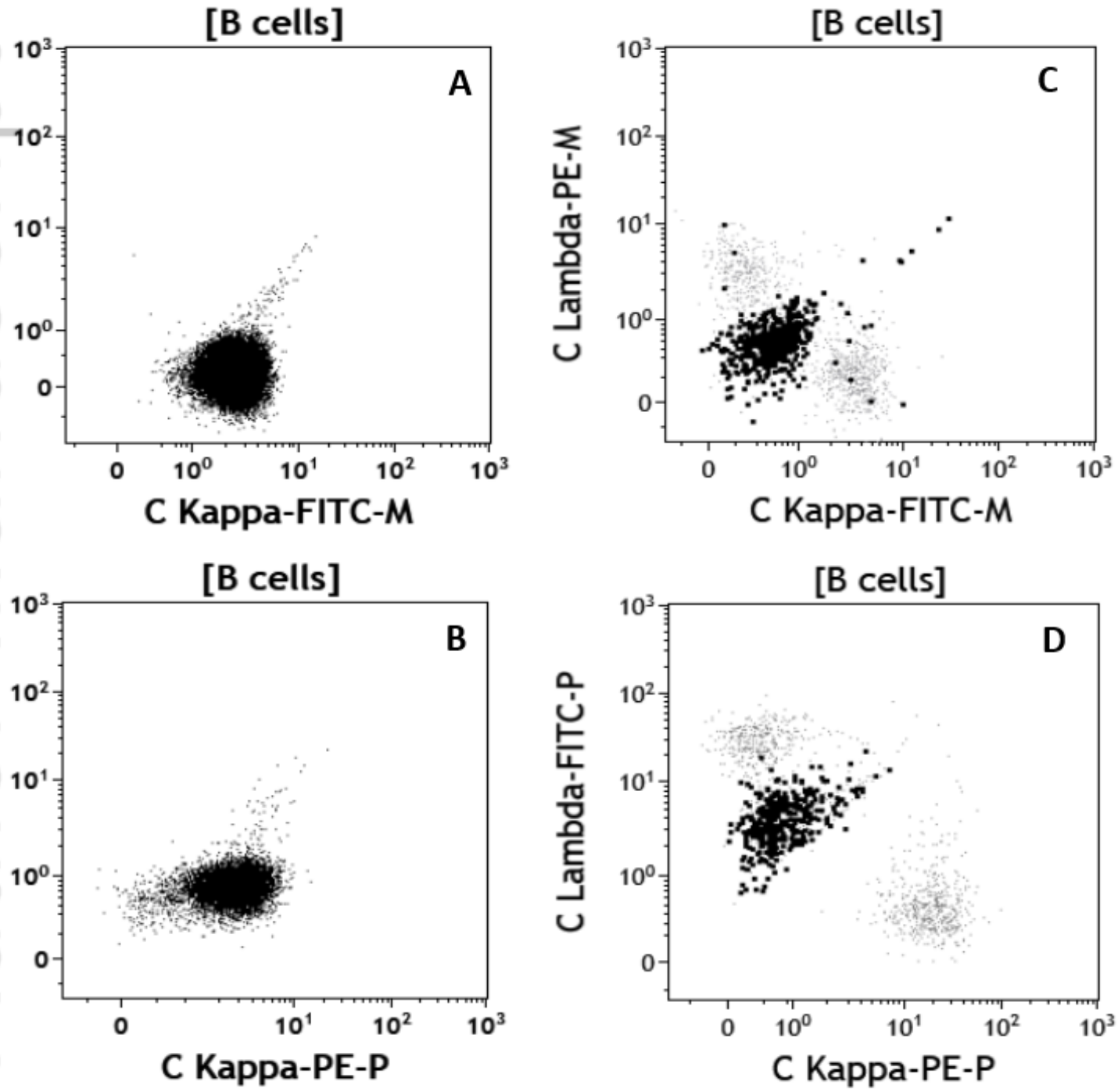
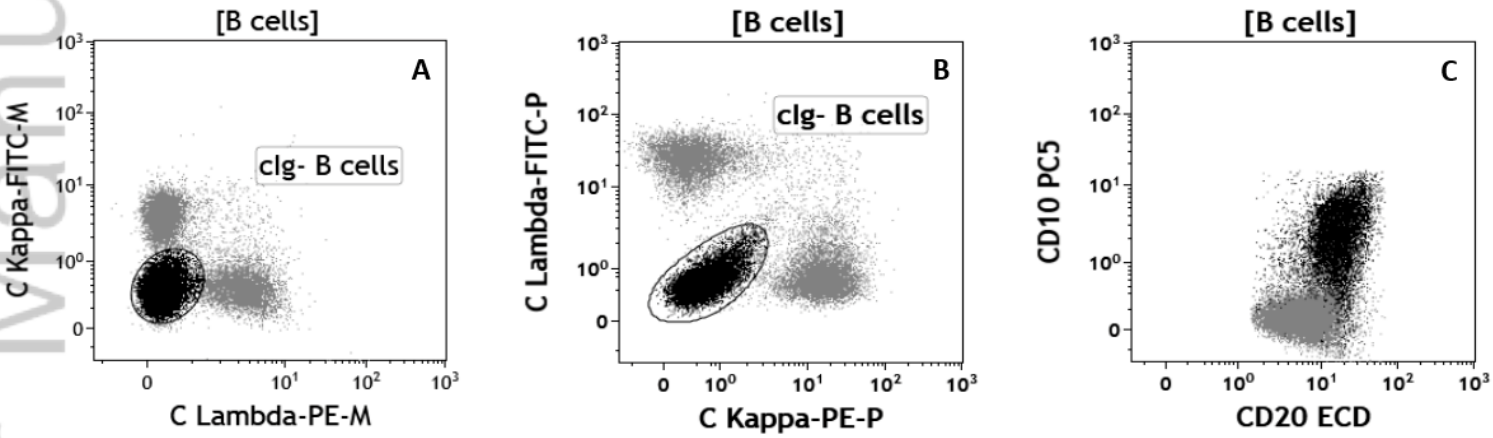


CYTOB_22107_Figure 3_R2.PNG



CYTOB_22107_Figure 4_R2.PNG