Significance of CD10-positive clonal B cell populations identified by flow cytometry in histologically benign gastric biopsies

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Abstract Historically, the presence of clonal B cell populations has been associated with malignancy in lymphoid proliferations. However, several studies have demonstrated that clonal B cell populations can be present in histologically reactive settings, but this has not been described in gastric samples. These studies have primarily shown clonality using molecular methods, with only a few studies showing clonality by flow cytometry. We evaluated 16 histologically benign gastric biopsies with CD10+ B cell populations identified by flow cytometry (13 clonal, 3 with kappa skew) and reviewed the morphology, endoscopic findings, and clinical course. Although the presence of clonal populations initially raised a diagnostic possibility of lymphoma in each case, morphologic examination supported a non-neoplastic reactive process. The CD10+ B cell populations typically demonstrated increased or slightly increased CD38 (88 %), increased or slightly increased CD20 (81 %), decreased surface light-chain expression (69 %), normal CD19 (94 %), and those that were studied ($n = 12$) were BCL-2 negative or indeterminate by flow cytometry. Polyclonal B cells were present, and the CD10+ B cell population comprised less than 50 % of the B cells in all cases. The available clinical information revealed no evidence of lymphoma in all 16 cases (follow-up ranging from 2 months

to 10 years). The presence of a CD10+ B cell clone (or light-chain skew) by flow cytometry in a gastric biopsy is not specific for lymphoid neoplasia and should be interpreted in the context of other immunophenotypic features as well as clinical and morphologic data.

Keywords CD10 positive · Clonal B cell population · Flow cytometry · Gastric lymphoma · Reactive gastritis

Introduction

Follicular lymphoma (FL) rarely arises in the stomach, whereas marginal zone lymphoma (MZL) of mucosa-associated lymphoid tissue (MALT) type is the predominant low-grade B cell lymphoma affecting the stomach [1–4]. Gastric lymphomas may present with symptoms such as dyspepsia and upper gastrointestinal bleeding and endoscopically may appear as nodularity, mass-like lesions and/or mucosal ulcers associated with varying degrees of mucosal thickening and vascularity [1, 2, 5]. Despite its rarity, FL of the stomach may be a consideration in the differential diagnosis of atypical lymphoid infiltrates in gastric biopsies, particularly if flow cytometric studies demonstrate a monotypic or clonal CD10+ B cell population [6]. Furthermore, reactive gastritis can mimic these low-grade lymphomas, creating diagnostic difficulty in correctly classifying atypical gastric lymphoid infiltrates. Early-stage MZL is typically treated with antibiotics (although may include radiation and chemotherapy), where FL patients would not be treated with antibiotics and may receive more aggressive therapy [4, 7, 8]. Although specific phenotypic (as defined by flow cytometry and immunohistochemistry (IHC)) and genotypic features can significantly increase the accuracy of lymphoma diagnosis, the morphologic assessment remains the cornerstone of diagnosis.

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Studies have demonstrated that clonal B cell populations can be identified not only in gastric lymphomas but also in reactive gastritis via immunophenotyping and/or molecular techniques [9–11], although some authors would argue otherwise [12]. Therefore, the usefulness of using molecular studies to identify monoclonal B cell populations in distinguishing benign lymphoid infiltrates from malignant lymphoid neoplasm may be limited. Similarly, Kussick et al. demonstrated that light-chain-restricted B cell populations of follicle center type (with low- to intermediate-level CD10 expression) can be identified by flow cytometry and/or molecular methods in histologically benign lymph node and tonsillectomy specimens [13]. However, there are no reports in the literature that have specifically addressed the detection of a CD10+ clonal B cell population by flow cytometry in histologically benign gastric biopsies and its potential implications.

In this regard, we describe 16 cases of histologically benign gastric biopsies that demonstrated CD10+ clonal (or light chain skewed) B cell populations identified by flow cytometry, illustrating the diagnostic complexities encountered when phenotypic abnormalities raise the question of neoplasia. In one of the cases studied, the population size by flow cytometry was large enough to raise a high level of concern for neoplasia (greater than 30 % of white cells). Therefore, we sought to determine the clinical behavior of CD10+ clonal (or light chain skewed) B cell populations seen in the stomach and to identify the morphologic, flow cytometric, and/or endoscopic findings that can help distinguish reactive CD10+ clonal/skewed B cell populations from bona fide lymphoid neoplasms (including MZL and FL).

Materials and methods

Patients and data collection

Using our pathology information system (Power Path) and Hematopathology flow cytometry database, 16 histologically benign gastric biopsies that contained CD10+ clonal/skewed B cell populations were identified at the University of Washington Hematopathology Laboratory between 2000 and 2014. Hospital electronic medical records were retrospectively reviewed for these patients to collect additional clinical information, including prior history of lymphoma, endoscopic findings, follow-up gastric biopsies (or lack thereof), possible causes of gastritis (such as *Helicobacter pylori* infection), and if there was evidence of new or recurrent lymphoma in the available clinical history. As comparison cohorts, the gastric biopsies with CD10+ clonal/skewed B cell populations were compared to bona fide low-grade B cell lymphomas with flow cytometry (including ten MZLs of the stomach and seven FLs of MALT sites). Our study was approved by the University of

Washington Institutional Review Board for human subjects research, #48070.

Flow cytometry

Fresh tissue was collected in Roswell Park Memorial Institute (RPMI) medium and processed for flow cytometry as described elsewhere [13]. Flow cytometry was performed as part of the clinical evaluation, using either a modified four-laser, ten-color Becton Dickinson LSR II flow cytometry (BD Biosciences, San Jose, CA) and prior to 2005 either a four-color FC-500 or Coulter Epics XL instrument (Beckman Coulter, Brea, CA). The antibody panels performed are listed in Table 1. At least 7000 events were acquired for the B cell analysis in all cases, with >20,000 events acquired in the majority. Data compensation and analysis were performed using software developed in-house (WoodList version 2.7 developed by Dr. Brent Wood, University of Washington Medical Center). Flow cytometric data was independently analyzed by two hematopathologists (L.S. and S.C.) to retrieve pertinent data, including the expression of light chain (including intensity and kappa/lambda ratio) as well as expression and intensity of CD5, CD10, CD19, CD20, CD38, and BCL-2. Percentage of CD10+ B cells (as compared to the numbers of total B cells or white cells) was also assessed. Flow cytometric evidence of clonality was determined using criteria reported by others (reactive germinal centers (GCs) showing a kappa/lambda ratio of 1.1–3.07) [14]; however, given the lack of consensus and reported variation [15], we opted to use a kappa/lambda ratio of ≤ 0.3 and ≥ 5 as evidence of clonality and 0.31–0.5 and 3.5–4.9 as evidence of skew. Antigenic intensity on the population of interest was assessed in comparison to the associated polyclonal non-GC B cells in the sample using visual inspection of the mean. Markers were considered slightly increased if the mean of the population in question was greater than the mean of the associated polyclonal B cells, but the populations were still overlapping and increased if there was no significant overlap (with the same criteria for decreases in intensity). Light chain was considered dim if the visual mean of the CD10+ population was less than that of the polyclonal, CD10-negative B cells. BCL-2 was considered positive if the population clearly showed greater intensity than the associated CD10-negative polyclonal B cells and T cells.

Morphology

The gastric biopsies were independently reviewed by two hematopathologists (L.S. and S.C.) to confirm the histological diagnosis and identify pertinent histological characteristics, including the presence of GCs, lymphoepithelial lesions (LEL), and the degree (mild, moderate, or severe) and pattern (diffuse, aggregate/follicular, or both) of lymphocytic

Table 1 Flow cytometry panel: antibodies, fluorochromes, and vendor

B cell panel	CD5: PE-Cy5.5 ^b	CD10: APC ^a	CD19: PE-Cy7 ^b	CD20: V450 ^a	CD38: A594 ^a	CD45: APC-H7 ^a	Kappa: FITC ^a	Lambda: PE ^a
BCL-2 panel	CD5: PE-Cy5.5 ^b	CD10: PE ^a	CD19: PE-Cy7 ^b	CD38: A594 ^a	BCL-2: FITC ^c			

Note that hyphenated fluorochromes are tandem conjugates

FITC fluorescein isothiocyanate, *PE* phycoerythrin, *Cy* cyanine dye, *APC* allophycocyanin, *APC-H7* allophycocyanin-hilite7, *V450* Violet 450 nm, *A594* Alexa 594 nm

^a Vendor: Becton Dickinson

^b Vendor: Beckman Coulter

^c Vendor: DakoCytomation

infiltrates. Multiple hematoxylin and eosin (H&E) levels were evaluated in all cases, and IHC studies were performed as part of the diagnostic work-up in some of the cases (IHC evaluated but not reported).

Results

Flow cytometric characteristics of gastric CD10+ clonal B cell populations and correlation with morphological and endoscopic findings

All 16 cases (13 patients) in this series are summarized in Table 2. The gastric biopsies came from five males and eight females ranging in age from 23 to 71 years (mean, 42 and 50 years, respectively). *H. pylori* infection was detected in Giemsa and H&E stains in 5 (31 %) of the 16 cases. Drug and infection were listed as possible etiologies of gastritis in the remaining cases, without histological evidence of *H. pylori*. Four patients had a prior history of lymphoma (two

with gastric MZL, one with splenic MZL, and one with gastric large B cell lymphoma (LBL)). Both gastric MZL cases had no flow studies but were both CD10 and BCL-6 negative by IHC. The splenic MZL was CD10 negative by flow cytometry, and the gastric LBL was reported from an outside institution without mention of flow cytometry or GC marker expression. In 13 cases, a surface light-chain-restricted B cell population was identified in gastric biopsies, with three cases demonstrating a light-chain skew (using the previously stated criteria for clonality). Eleven (69 %) of the 16 cases demonstrated kappa light-chain restriction or skew, whereas the remaining five cases (31 %) showed lambda light-chain restriction or skew. A majority of cases (69 %) showed dim intensity of light-chain expression as compared to background non-GC B cells, as described by others in reactive scenarios [14, 16]. The mean percentage of clonal/skewed CD10+ B cells was estimated to be 16.2 % (min = 4.2 %, max = 47 %) among all B cells and 6.3 % (min = 0.3 %, max = 34.3 %) among all white cells. All light-chain-restricted/skewed B cell populations demonstrated the characteristic follicle-center-

Table 2 Flow cytometric and morphological findings of CD10+ clonal B cell populations in histologically benign gastric biopsies

Case no.	Age/sex (follow-up)	B cell κ/λ ratio	BCL-2	CD19	CD20	CD38	Estimated clonal/skewed B cells (% of B cells)	Estimated clonal B cells (% of white cells)	GC	LEL	Degree of infiltration	Pattern of infiltration
1	46/F (2 months)	κ (dim, 3.8)	Neg	Normal	Normal	Increased	10.6	2	Yes	No	Mild	Aggregate
2	63/F (1 year)	κ (147)	Neg	Normal	Increased	Increased	37.9	17.1	No	No	Mild	Aggregate
3	30/M (2 years)	κ (dim, 23)	Neg	Slightly increased	Increased	Increased	44.4	4.4	No	No	Mild	Aggregate and diffuse
4	61/F (2 years)	λ (0.08)	ID	Normal	Normal	Normal	4.3	1.1	No	No	No	No
5	30/M (4 years)	λ (0.04)	Neg	Normal	Slightly increased	Increased	6.1	2.4	Yes	No	Mild	Aggregate
6	33/F (5 months)	κ (dim, 15.8)	Neg	Normal	Increased	Increased	12	4.2	No	No	Mild	Aggregate
7	69/M (5 years)	κ (dim, 5.4)	TFE	Normal	Slightly increased	Increased	7.2	3.4	Yes	No	Mild	Aggregate
8	23/M (2 years)	λ (dim, 0.02)	Neg	Normal	Increased	Increased	4.2	0.3	No	No	Mild	Aggregate
9	59/M (1 year)	κ (dim, 12.5)	Neg	Normal	Normal	Increased	12.2	5.9	Yes	No	Moderate	Aggregate and diffuse
10	61/F (6 years)	λ (dim, 0.4)	Neg	Normal	Increased	Increased	15.4	8.3	Yes	No	Moderate	Aggregate
11	58/F (1 year)	λ (0.04)	ND	Normal	Slightly increased	Normal	8.3	0.35	No	No	Moderate	Aggregate and diffuse
12	71/F (10 years)	κ (dim, 10.1)	TFE	Normal	Slightly increased	Increased	5.9	1.3	No	No	Mild	Aggregate
13	57/F ^a (10 years)	κ (dim, 59)	Neg	Normal	Slightly increased	Increased	10.6	0.7	No	No	Mild	Aggregate
14	54/F ^a	κ (dim, 7.1)	Neg	Normal	Increased	Slightly increased	16	8.8	Yes	No	Moderate	Aggregate
15	54/F ^a	κ (dim, 3.9)	Neg	Normal	Slightly increased	Slightly increased	16.8	5.7	Yes	No	Moderate	Aggregate
16	54/F ^a	κ (9.7)	ND	Normal	Increased	Increased	47	34.3	Yes	No	Moderate	Aggregate and diffuse

^a The same patient with multiple cases/time points of assessment

ID indeterminate, *ND* not done, *TFE* too few events, *Neg* negative, *GC* germinal left, *LEL* lymphoepithelial lesion

associated CD10 expression. In addition, as reported by others [13, 14, 17, 18], we noted more consistent, slightly increased to increased CD20 and increased CD38 expression in 13 (81 %) and 14 (88 %) of the 16 cases, respectively, when compared to the associated polyclonal CD10-negative B cells (Fig. 1a, b). Figure 2 additionally demonstrates cases with “normal” or “slightly increased” CD20 and/or CD38 expression in comparison to a case demonstrating “increased” CD20 and CD38 expression. The levels of CD19 in 15 (94 %) of the 16 clonal populations were similar to those of the background polyclonal B cells. There was no evidence of CD5 expression or BCL-2 overexpression in all cases examined (16 and 11 cases, respectively)

The morphologic examinations of all 16 cases showed mild to moderate lymphocytic infiltrates with a predominantly nodular architectural pattern in 15 of 16 (94 %), a subset of which showed an additional diffuse component (5 of 15; 33 %) (Fig. 3a–d). One case demonstrated no appreciable lymphoid infiltrate. There were no LELs in all cases. GCs were identified in 8 (50 %) of the 16 cases (histologically composed of polymorphous lymphoid cells with tingible body

macrophages and mitotic activity, without follicular effacement by a uniform population of small cleaved cells). Upper endoscopy demonstrated mucosal thickening/irregularity and/or erosions/ulcers in seven cases. Only one case had both mucosal ulcers and associated mucosa thickening, which are common gross findings in patients with gastric lymphomas [2]. No mass-like lesions were identified. No patients developed lymphoma based on the available clinical (and, in some cases, histologic) follow-up (clinical follow-up 2 months to 10 years with average follow-up of 3.4 years).

Comparison of gastric CD10+ clonal B cell populations to histologically proven low-grade B cell lymphomas

As comparison cohorts, the gastric biopsies with CD10+ clonal B cell populations were compared to bona fide low-grade B cell lymphomas (including ten gastric MZLs and seven FLs from non-gastric, MALT sites). All gastric MZL cases were clonal (70 % kappa restricted) without expression of CD10 or CD5 (Table 3), and six of the ten cases showed normal CD20 (three increased/slightly increased and one decreased). The

Fig. 1 Dot plots showing a reactive clonal CD10+ population (a, b) in comparison to follicular lymphoma at mucosal/extranodal sites (c, d). CD10+ B cells are highlighted in yellow (and emphasized if <50 % of the total B cells) and polyclonal B cells highlighted in blue. Notice the normal CD19 and increased CD20 and CD38 expression in the reactive clonal CD10+ B cell populations (arrows, a, b) versus decreased CD19, normal or decreased CD20, and normal CD38 in the follicular lymphoma (arrows, c, d)

Fig. 2 Dot plots of all lymphocytes. The clonal or skewed CD10+ population is *highlighted* and emphasized in *yellow* (arrowed population); the CD19+, CD10-negative polyclonal B cells are *highlighted* in *blue*, and T cells are *highlighted* in *green*. The reactive, clonal CD10+ B cell population with the typical increase in CD20 and CD38 expression (**a**, case 2 from Table 2) can be compared to a case with only slightly increased CD20 and CD38 (**b**, case 15), a case with slightly increased CD20 and normal CD38 (**c**, case 11), and a case with normal CD20 and increased CD38 (**d**, case 1). The antigens that were considered “slightly increased” show some overlap with the CD10-negative B cells, although show a slightly increased shift in antigen intensity

Fig. 3 Morphologic examination of reactive gastritis with a clonal CD10+ population identified by flow cytometry (case 2 from Table 2). **a** A mild to moderate lymphocytic infiltrate is seen with a predominantly nodular architectural pattern (H&E stain, ×2). **b** CD20 stain highlights B cells (×2), some areas more numerous than CD3. **c** CD3 stain highlights T cells, which are less numerous than the B cells, but not markedly less (×2). **d** Lymphocytes in reactive gastritis tend to have round nuclear contours without abundant cytoplasm, and no lymphoepithelial lesions are seen (H&E, ×20)

levels of CD19 and CD38 in all ten clonal populations were similar to those of the polyclonal B cells in the background (normal expression level). The mean percentage of the clonal population was relatively high in gastric MZLs, representing 84 % (min = 60 %, max = 98 %) among all B cells and 40 % (min = 10 %, max = 87 %) among all white cells. Consistent with the greater mean percentage of clonal populations identified by flow cytometry, all cases showed moderate to severe lymphocytic infiltrates in a diffuse architectural pattern with the presence of LELs in nine (90 %) of the ten cases (Fig. 4a–d). No GC was identified in nine (90 %) of the ten cases. Endoscopically, MZL showed mucosal thickening/erosion/ulcerations in 90 % of the cases, with one of these patients demonstrating a mass-like, linitis plastica appearance.

Due to the rarity of gastric FL, seven FL cases from non-gastric sites (including two duodenal, one lung, two parotid, and two orbital) were analyzed for comparison (Table 4). As expected, all FL cases were composed of clonal or light-chain-negative, CD10+ B cell populations. The clonal populations demonstrated normal to decreased CD20 expression in six of seven cases (86 %), and CD19 was variable (three normal, three decreased, and one increased) (Fig. 1c, d). All cases

Table 3 Flow cytometric and morphological findings of clonal B cell populations in gastric marginal zone lymphomas

Case no.	Age/sex	B cell κ/λ ratio	CD10	CD19	CD20	CD38	Estimated clonal B cells (% of B cells)	Estimated clonal B cells (% of white cells)	GC	LEL	Degree of infiltration	Pattern of infiltration
1	77/M	λ (0.03)	No	Normal	Increased	Normal	88	55	Yes	Yes	Severe	Diffuse
2	71/M	κ (138)	No	Normal	Normal	Normal	70	20	No	Yes	Severe	Diffuse
3	79/M	λ (0.23)	No	Normal	Increased	Normal	85	23.20	No	No	Severe	Diffuse
4	64/F	λ (0.035)	No	Normal	Normal	Normal	95	12	No	Yes	Severe	Diffuse
5	58/M	κ (5.6)	No	Normal	Decreased	Normal	60	38.40	No	Yes	Severe	Diffuse
6	43/M	κ (32)	No	Normal	Normal	Normal	91	56.40	No	Yes	Moderate	Diffuse
7	37/F	κ (14.5)	No	Normal	Normal	Normal	81	65.30	No	Yes	Severe	Diffuse
8	87/F	κ (142)	No	Normal	Slightly increased	Normal	98	87	No	Yes	Severe	Diffuse
9	69/F	κ (18)	No	Normal	Normal	Normal	85	10.30	No	Yes	Severe	Diffuse
10	35/F	κ (82)	No	Normal	Normal	Normal	86	28.10	No	Yes	Severe	Diffuse

GC germinal left, LEL lymphoepithelial lesion

showed normal CD38 expression. These patterns of expression are similar to what has been reported previously [18, 19], including decreased expression of CD19 and normal expression of CD38 (decreased in comparison to follicular hyperplasia). The reported expression of CD20 in FL has been more variable. BCL-2 expression was assessed and positive in two cases.

Fig. 4 Morphologic examination of gastric marginal zone lymphoma (case 2 from Table 3). **a** A moderate to severe lymphocytic infiltrate is seen with a diffuse architectural pattern (H&E stain, $\times 2$). **b** CD20 stain highlights numerous B cells ($\times 2$). **c** CD3 stain highlights T cells that are much less numerous than the B cells and less than that seen in reactive gastritis ($\times 2$). **d** Lymphocytes have more irregularity to their nuclear membranes, and some have more abundant cytoplasm than seen in reactive gastritis. Lymphoepithelial lesions (LELs) are present (H&E, $\times 20$, with insert of CD20 LEL (circle))

Discussion

Our findings demonstrate that CD10+ clonal (or light chain skewed) B cell populations can be detected by flow cytometry in histologically benign gastric biopsies. Although these cases are not common (only 16 cases were identified during the 14-year period from 2000 to 2014), the true incidence is uncertain, as concurrent flow cytometric studies are not typically performed in patients without a clinical suspicion of gastric lymphoma (samples are taken for flow based on prior history of lymphoma, prior histologic findings worrisome for a lymphoid neoplasm, clinical symptoms, and/or endoscopic/imaging findings). All cases included in this study had been previously evaluated by morphology (some with immunohistochemical studies), and diagnosed as, or favored to be reactive. To verify the original diagnoses, the histological features of each case were independently reviewed by two hematopathologists (L.S. and S.C.), who concurred with the original “reactive” diagnoses. Furthermore, the review of subsequent gastric biopsies (or lack thereof) and the available clinical information demonstrate that none of these patients have developed new and/or recurrent lymphoma (clinical follow-up 2 months to 10 years). Taken together, these findings support the notion that CD10+ clonal B cell populations in histologically benign gastric lymphoid proliferations may represent reactive clonal hyperplasia of follicle center B cells rather than an underlying lymphoma, similar to clonal populations that have previously been published in lymph nodes with reactive follicular hyperplasia and in the thyroid in Hashimoto thyroiditis [13, 20]. In fact, this is

Table 4 Flow cytometric findings of clonal B cell populations in mucosa-associated follicular lymphomas

Case no.	Age/sex	Biopsy site	Light chain	CD10	CD19	CD20	CD38	BCL-2	Estimated clonal B cells (% of B cells)	Estimated clonal B cells (% of white cells)
1	66/M	Duodenum	κ	Yes	Nml	Nml	NR	ND	NR	43
2	50/F	Duodenum	λ (0.07)	Yes	Increased	Increased	Nml	ND	92	30
3	50/F	Lung	λ (0.004)	Yes	Nml	Subset decreased	Nml	ND	99	64
4	60/M	Parotid	sIg negative	Yes	Decreased	Nml	Nml	Pos	38	20
5	87/F	Orbit	sIg negative	Yes	Decreased	Nml	Nml	ND	15 ^a	8
6	73/M	Parotid	sIg negative	Yes	Decreased	Decreased	Nml	ND	59 ^a	40
7	61/M	Orbit	λ	Yes	Nml	Decreased	Nml	Pos	NR	NR

Assessment of kappa/lambda ratio only possible in patients 2 and 3

sIg surface light chain, ND not done, NR not reported, Nml normal or similar to background B cells, Pos positive

^a Patient also had concurrent chronic lymphocytic leukemia/small lymphocytic lymphoma

consistent with the previous report that a polyclonal set of B cells, upon activation by antigen, may go through multiple rounds of proliferation and mutation, ultimately leading to a few large dominating B cell clones after elimination of unselected cells [21]. As such, the presence of monoclonal CD10+ B cells identified by flow cytometry should be correlated with morphologic, clinical, and potentially cytogenetic/molecular findings before making a final diagnosis. Although it is unclear as to what antigens are responsible for activating monoclonal CD10+ B cells, immunologic response to *H. pylori* infection and/or a prior history of lymphoma may play a role, as approximately 30 % of the cases had one of these potential risk factors.

Features associated more commonly with overt lymphoma in a CD10+ population include a high percentage of clonal cells (greater than 50 % of all B cells), decreased expression of CD19, and normal CD38 expression (Fig. 1c, d). This is in agreement with the previous reports that FL often aberrantly expresses CD20 and/or CD19 (typically decreased) with normal (or decreased in comparison to follicular hyperplasia) CD38 [19, 22]. By contrast, the reactive clonal B cells of follicular hyperplasia more consistently display slightly increased to increased CD20 and CD38 expression, with normal CD19 (Fig. 1a, 1b), also similar to that previously reported in lymph nodes [14, 17, 18]. The clonal but reactive population in this setting typically represents less than 20 % of all B cells (81 % of cases in this study) or less than 10 % of all white cells (88 % of cases in this study). Flow cytometric evaluation of BCL-2 overexpression is very useful in such cases, since increased BCL-2 expression is detectable in a majority of FL cases, whereas reactive follicular lymphoid hyperplasia consistently shows negative (or rarely indeterminate) BCL-2 staining by flow cytometry [2, 17, 23–26]. Indeed, the CD10+ clonal GC B cell populations studied in our series did not overexpress BCL-2 nor have the patients developed lymphoma based on the review of subsequent gastric biopsies and the available clinical information.

Furthermore, the review of morphologic and endoscopic findings demonstrates that the presence of mild to moderate lymphocytic infiltrates having a nodular/lymphoid aggregate architectural pattern and/or the presence of GCs (without evidence of FL in situ) supports a benign diagnosis (Fig. 3a–d), whereas the gastric MALT lymphomas in this study typically showed diffuse, moderate to severe lymphocytic infiltrates with frequent LELs and lack of GCs (Fig. 4a–d) [2, 27]. Although GCs were seen in only 50 % of cases (that would typically correspond to a CD10+ B cell population in a benign setting), the findings suggest that there was variation in the cellular components present in the tissue sampled for flow cytometry versus the tissue sampled for H&E examination (multiple H&E levels were morphologically evaluated).

In conclusion, the data presented here provides important information regarding the management of CD10+ clonal B cell populations identified by flow cytometry and the importance of correlation with the morphologic and clinical findings. The findings support conservative clinical management for patients with CD10+ clonal B cell populations identified by flow cytometry but benign histology and reactive rather than aberrant immunophenotypic features, as available clinical follow-up revealed no evidence of lymphoma in all 16 cases. Interesting future directions could include evaluation of the mutational landscape of both reactive and neoplastic gastric processes, as well as evaluation for these clonal (or skewed) CD10+ B cell populations in other parts of the gastrointestinal tract.

Compliance with ethical standards All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. For this type of study, formal consent is not required.

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Conflict of interest The authors declare that they have no conflicts of interest.

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