Monoclonal gammopathies regardless of subtypes are associated with poor prognosis of diffuse large B-cell lymphoma
A STROBE-compliant article
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Abstract
Monoclonal gammopathy (MG), a positive result of serum immunofixation electrophoresis (SIFE), has been reported in cases of diffuse large B-cell lymphoma (DLBCL). We performed this study to further investigate the prognostic value of MG in DLBCL.

We retrospectively reviewed patients diagnosed with DLBCL between January 2007 and December 2014, and identified 37 patients with MG. The clinical characteristics of these patients were then reviewed. A 1:2 case-control analysis was conducted on 74 matched controls, who were patients with DLBCL and without MG. Both cases and controls were age-matched and were diagnosed within the same year.

Among 37 DLBCL patients with MG, the monoclonal component of IgM was the most frequent compared to the other subtypes. Laboratory tests showed that the presence of MG was correlated with a decreased platelet-to-lymphocyte ratio (PLR). Survival analysis showed that MG-secreting DLBCL patients had an inferior overall survival (OS) and progression-free survival (PFS), compared with MG-nonsecreting patients, regardless of MG subtype. However, treatment response analysis showed that MG was not a good indicator for tumor relapse. When patients with DLBCL were grouped by immunophenotype, we found that MG was associated with poor prognosis in the non-germinal center B-cell-like (GCB) type, rather than GCB type in OS analysis. Meanwhile, there was no statistical significance upon PFS analysis in both immunophenotypes. Furthermore, our study found that the appearance of MG during treatment did make prognostic sense compared to nonsecretors.

Overall, MG can serve as a prognostic factor for DLBCL. We hypothesize that its presence in DLBCL may reflect the immune microenvironment in tumor progression and warrants further study to unveil the underlying molecular pathogenesis.

Abbreviations: CR = complete remission, DLBCL = diffuse large B-cell lymphoma, FLC = free light chain, GCB = germinal center B-cell-like, IPI = international prognostic index, LMR = lymphocyte-to-monocyte ratio, MG = monoclonal gammopathies, NLR = neutrophil-to-lymphocyte ratio, OS = overall survival, PCD = plasma cell diseases, PFS = progression-free survival, PLR = platelet-to-lymphocyte ratio, PR = partial remission, SIFE = serum immunofixation electrophoresis.

Keywords: diffuse large B-cell lymphoma, monoclonal gammopathy, prognosis, serum immunofixation electrophoresis

1. Introduction
Diffuse large B-cell lymphoma (DLBCL) is the most common pathologic type of high-grade non-Hodgkin lymphoma among adults, with an estimated annual incidence of 7 to 8 cases per 100,000 people every year.[1,2] Standard treatment for DLBCL includes a combination of biological therapy consisting of rituximab and anthracycline-based chemotherapy regimens, usually comprising cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP). Owing to the use of rituximab, progression-free survival (PFS) and overall survival (OS) rates in DLBCL have improved.[3] However, the International Prognostic Index (IPI), which was developed in the era of chemotherapy treatment for aggressive lymphoma, has proven to be less powerful in the rituximab era. To better distinguish tumor prognosis, several models[4,5] and algorithms[6,7] have been developed. In addition, several markers have been discovered in DLBCL, to better identify patients who will likely have a poor therapeutic response.[8] Despite the rise of interests in these areas, there is still a strong need to discover more prognostic factors based on tumor phenotypes that will probably improve risk-adapted treatment approaches.

Serum immunofixation electrophoresis (SIFE) technology is used to identify monoclonal or polyclonal gammopathies.[9] Compared to serum free light chain (FLC) testing, which is becoming a mainstream screening test for plasma cell diseases (PCD), SIFE remains a lower-cost test, and is more widely used in clinical practice. Usually, a positive SIFE result refers to the discovery of serum monoclonal gammopathies (MG), and such results are mainly observed in patients with multiple myeloma.
1.4 mg/m² vincristine on day 1 and 100 mg prednisolone on days 1–5. Patients with bone marrow, genital organ, glands or craniofacial site involvement, or with involvement of ≥2 extra nodal sites, received 4 to 6 injections of 12 mg methotrexate for intrathecal prophylaxis. The International Workshop Criteria for non-Hodgkin lymphoma was used to evaluate the patients’ response to treatment and the incidence of relapse. These patients also received physical examinations, blood tests, and computed tomography (CT) or positron emission tomography-CT scans to monitor their disease status during follow-up. The PFS was calculated as the length of time from diagnosis to disease progression, relapse, or death of any cause. The OS was calculated as the time from diagnosis to death because of any cause. Patients without an event or death were censored at the time of last known follow-up.

2. Methods

This study was designed as a retrospective 1:2 case-control study.

2.1. Study subjects and selection criteria

This case-control study evaluated the incidence of patients with MG-secreting DLBCL, compared to a group of MG-nonsecreting controls. Clinicopathological features, treatment response, and survival were compared between the 2 groups. The study was approved by the Ethics Review Committee, Zhongshan Hospital of Fudan University, Shanghai, China. All patients provided written consent for the use of their clinical data, including laboratory test results. These results were retrospectively collected and obtained from corresponding medical records.

Thirty-seven patients were diagnosed with MG-secreting DLBCL between January 2007 and December 2014, according to the World Health Organization criteria. They were analyzed for SIFE throughout disease onset, middle of the treatment, and follow-up. As controls, a set of 74 MG-nonsecreting patients with DLBCL (1:2) were selected under the same diagnostic criteria. An eligible control was matched to a case by age (±5 years) and within the same year of diagnosis. Usually, >2 patients met the matching criteria, in which case we selected 2 matched individuals with similar Ann Arbor stage, and IPI score. Lastly, if >2 patients met the matching criteria, 2 matched individuals were selected randomly.

2.2. Immunohistochemistry

Cell of origin was assessed by immunohistochemistry according to the Hans algorithm. Based on the 3 markers, CD10 (56 C6, Novacastra/Leica microsystems, Wetzler, Germany), BCL6 (PG-B6p, DAKO, Glostrup, Denmark) and MUM1 (MUM1p, DAKO, Glostrup, Denmark), according to the Hans classifier, the subgroups are as follows: GCB type (CD10+/CD10– BCL6+/MUM1–), non-GCB type (CD10– BCL6–MUM1+/CD10– BCL6–). All biopsies were reviewed by 3 pathologists. The conditions for all the antibodies and their evaluation followed the guidelines recommended by the Lymphoma Biomarker Consortium.

2.3. Treatment, treatment response evaluation, and survival time

All patients enrolled in the study received 6 to 8 cycles of R-CHOP every 21 days (375 mg/m² rituximab on day 0, 50 mg/m² doxorubicin on day 1, 750 mg/m² cyclophosphamide on day 1, 1.4 mg/m² vincristine on day 1 and 100 mg prednisolone on days 1–5).
these patients then became SIFE-positive during the treatment, whereas 6 patients who were initially SIFE-positive became negative during the treatment. These 19 patients matched with themselves in pairs according to SIFE status, and were further evaluated for NLR, LMR, and PLR. We found that the levels of PLR were associated with the change of SIFE results (Fig. 1B). Those SIFE-positive patients with DLBCL tend to have low PLR. When SIFE results indicated that it was negative, the level of PLR increased accordingly ($P = 0.016$).

### 3.3. MGs as a prognostic factor for DLBCL

Median follow-up was close to 12 months for the MG-secreting patients with DLBCL (range 2–84 months). As shown in Figure 2, the median OS was 60 months (95% CI 17.32–102.67) in the MG-secreting DLBCL group compared to 86.54 months (95% CI 66.67–107.33) in the MG-nonsecreting group ($P = 0.008$). Three-year OS rates were 60.5% (95% CI 51.7–69.3) in the MG-secreting DLBCL group, and 84.8% (95% CI 79.3–90.3) in the nonsecreting group ($P = 0.001$). The median PFS for secreting group was 44.89 months (95% CI 31.08–58.71) compared to 84 months (95% CI 62.72–93.26 months) in the nonsecreting group ($P = 0.001$). At 3 years, the PFS rate was 52.3% (95% CI 41.8–62.8) in the MG-secreting group and 76.4% (95% CI 70–82.8) in the nonsecreting group ($P = 0.01$). Differences in the OS and PFS among the various MG subtypes did not reach statistical significance ($P = 0.953$ and $P = 0.952$ respectively for median OS and PFS). Moreover, having a positive SIFE present during the treatment did make prognostic sense as compared to the nonsecreting ones (median OS 42.6 vs. 86.54 months, $P = 0.004$).

### Table 1

|                          | Case (N=37) | Control (N=74) | OR (95% CI) | $P$  |
|--------------------------|-------------|----------------|-------------|------|
| Sex (male vs. female)    | 24 (64.9)   | 36 (45.9)      | 2.172 (0.961–4.908) | .06  |
| Age ($\geq 60$ vs. $<60$) | 21 (56.8)   | 52 (70.3)      | 1.801 (0.794–4.087) | .157 |
| Ann Arbor I–IV vs. III–IV | 10 (27)     | 32 (43.2)      | 0.4876 (0.206–1.148) | .097 |
| B symptom*               | 18 (48.6)   | 35 (47.2)      | 0.947 (0.430–2.067) | .893 |
| GCB vs. nonGCB           | 21 (56.8)   | 44 (60)        | 0.809 (0.359–1.807) | .599 |
| PI score (0–1 vs. 2–5)   | 8 (21.6)    | 24 (32.4)      | 0.575 (0.229–1.445) | .236 |

*D = confidence interval, GCB = germinal center B-cell like, PI = international prognostic index, N = number, non-GCB = non-germinal center B-cell like, OR = odds ratio.

*Systemic symptoms of fever, night sweats, and weight loss.

### Table 2

| Subtype | N  | PCT (%) |
|---------|----|---------|
| IgG     | 12 | 32.4    |
| IgG-λ   | 5  | 13.5    |
| IgG-κ   | 7  | 18.9    |
| Igλ     | 6  | 16.2    |
| Igλ-λ   | 3  | 8.1     |
| Igλ-κ   | 3  | 8.1     |
| Igμ     | 19 | 51.4    |
| Igμ-λ   | 10 | 27.0    |
| Igμ-κ   | 9  | 24.4    |

DLBCL = diffuse large B-cell lymphoma, N = number, PCT = percentage.

### Figure 1

Ratios of different kinds of peripheral blood cells and SIFE results. (A) NLR, PLR, and LMR are grouped by secreting and nonsecreting DLBCL. The bars represent the mean ± standard deviation. (B) Among 37 MG-secreting DLBCL, 13 initially SIFE-negative patients became positive and 6 initially SIFE-positive patients became negative during the treatment. These 18 patients were further evaluated for NLR, LMR, and PLR by compared t test according to SIFE results. SIFE = serum immunofixation electrophoresis, NLR = neutrophil-to-lymphocyte ratio, LMR = lymphocyte-to-monocyte ratio, PLR = platelet-to-lymphocyte ratio.

**Figure 1.** Ratios of different kinds of peripheral blood cells and SIFE results. (A) NLR, PLR, and LMR are grouped by secreting and nonsecreting DLBCL. The bars represent the mean ± standard deviation. (B) Among 37 MG-secreting DLBCL, 13 initially SIFE-negative patients became positive and 6 initially SIFE-positive patients became negative during the treatment. These 18 patients were further evaluated for NLR, LMR, and PLR by compared t test according to SIFE results. SIFE = serum immunofixation electrophoresis, NLR = neutrophil-to-lymphocyte ratio, LMR = lymphocyte-to-monocyte ratio, PLR = platelet-to-lymphocyte ratio.
median PFS of 30.67 vs. 84 months, \( P = .008 \). However, the prognostic value of MG had its limitations in distinguishing survival outcomes between 2 MG-secreting subgroups \( P > .05 \). When patients were grouped by immunophenotype, our results further showed that MG was associated with poor prognosis in the nonGCB type, rather than GCB type for OS analysis (Fig. 3A and B). Although PFS analysis showed that nonsecreting DLBCL patients had longer OS and PFS time than secreting ones in both GCB and nonGCB types, the differences did not reach statistical significance (Fig. 4A and B).

In terms of treatment response, among the 37 patients with MG-secreting DLBCL that were evaluated, 10 patients (27.0\%) progressed after 3 cycles of first-line treatment, and a total of 23 patients (62.2\%) achieved complete remission (CR) or partial remission (PR) (13 patients reached CR and 10 reached PR). As for the 74 patients with nonsecreting DLBCL, 19 patients (25.7\%) progressed after 3 cycles R-CHOP treatment. The number of people who reached CR or PR was 44 (59.5\%). No significant difference was identified between the MG-secreting and nonsecreting groups for treatment response rate.

4. Discussion
William et al previously reported that FLC abnormalities were present in 13\% of 208 patients with lymphoma, and the prevalence of abnormal k:\( \lambda \) FLC ratio in DLBCL was about 8\%.\[14\] However, limited data have been reported on the exact prevalence of MG in different pathologic types of lymphoma, and there are few studies focusing on the molecular pathogenesis behind. We conducted a retrospective case-control study.
including 37 DLBCL patients with positive SIFE. Regarding the subtypes of MG, the monoclonal component of IgM was more frequent compared to other subtypes (51.4%) among these 37 patients. Contrary to our study results, Economopoulos et al reported that IgM-MG was more frequent in cases with low-risk NHL, such as that of mucosa-associated lymphoid tissue lymphoma, follicular lymphoma, among others, whereas IgG was more frequent in cases with aggressive NHL such as DLBCL, Burkitt lymphoma, and so on.

This discrepancy may be because of the limited number of patients in both studies. Further studies with larger sample size should be conducted in the future to find out whether MG subtypes correlate with lymphoma pathologic types.

Another study by Cox et al found that after 1 to 3 cycles of R-CHOP, the monoclonal IgM component disappeared in all SIFE-positive patients. Some patients were persistently negative for IgM either during continuous CR or after recurrence (75% patients), whereas some underwent the reappearance of a monoclonal IgM preceding disease relapse. In our study, among the 37 patients with MG-secreting DLBCL, the total number of people who reached CR or PR after 3 cycles of R-CHOP was 23 (62%). Among the 24 patients who were initially SIFE-positive, 6 became negative during treatment. Half of these 6 patients experienced disease progressions. Meanwhile, up to half of the patients who were initially SIFE-negative became positive while reaching persistent CR. As a result, the findings of both Cox et al, and our own findings reveal that MG, although has prognostic value, it might not serve as a proper monitor for both Cox et al, and our own positive while reaching persistent CR. As a result, the progression did not progress to lymphoma with time. Therefore, they concluded that rather than polyclonal gammopathies which are regarded as a reaction of the anti-tumor response, MG may be attributed to disease-related immune stimulation.

Recent studies have introduced ratios of different kinds of peripheral blood cells to be used to predict prognosis of lymphoma. Watanabe et al revealed that LMR can reflect host systemic immunity and estimate clinical effects of R-CHOP treatments for DLBCL patients. Wang et al found that a decreased LMR led to a weak anti-tumor immunity and could be used as a poor prognostic biomarker of DLBCL. Meanwhile, PLR was reported as a potential predictive factor for several malignancies and cardiovascular diseases. A lower PLR was reported to be associated with poor prognosis in a few types of lymphoma. In our study, we found that DLBCL patients who were SIFE-positive tend to have lower PLR. We suspected that the relationship of the low PLR and positive SIFE may be associated with the lymphoma immune environment and worth further investigation.

5. Conclusion
Overall, a variety of laboratory tests, including serum protein electrophoresis (SPEP), urine protein electrophoresis, SIFE, and serum FLC assays, can be performed to detect MG. It was reported that the presence of M-protein, discovered by SPEP or serum FLC, is associated with poor prognosis in patients with DLBCL. Previously, IFE was regarded as being specific but not sensitive. As a result, there is a possibility that we may potentially have missed some patients who have low-grade MG, which may partly explain the negative SIFE results before
treatment, which then subsequently became positive. Owing to the limited number of cases reported, further studies with a larger number of participants and longer follow-up are needed to clarify this observation. Finally, we conclude that MG, regardless of subtypes, could serve as a potential prognostic marker for DLBCL, and the molecular pathogenesis behind worth the effort to unveil it.

Author contributions

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