

Soluble CD27 Is a Faithful Marker of Disease Burden and Is Unaffected by the Rituximab-Induced IgM Flare, as Well as by Plasmapheresis, in Patients with Waldenström's Macroglobulinemia

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Abstract

Background: The assessment of disease burden is often difficult in patients with Waldenström's macroglobulinemia (WM) who receive rituximab due to the induction of an IgM flare, and following the removal of serum IgM by plasmapheresis. Soluble CD27 (sCD27) is a tumor necrosis factor family member secreted by WM cells which is strongly correlated with serum IgM levels and clinical responses in patients with WM. As such, we attempted to delineate its potential role in WM patients experiencing a rituximab-induced IgM flare and following plasmapheresis. **Patients and Methods:** sCD27 levels were serially measured by serum-based ELISA in 8 patients who ultimately demonstrated a response to therapy, and in whom a rituximab-mediated IgM flare was observed, as well as in 3 WM patients undergoing plasmapheresis. **Results:** Among the 8 patients who experienced a rituximab-mediated IgM flare, IgM levels rose from 3515 to a peak of 5270 mg/dL ($P = .008$), while sCD27 levels decreased from 174.1 to 155.9 U/mL ($P = .012$), with a decline observed in all patients. Among 3 patients undergoing plasmapheresis, IgM levels declined from a median of 6940 to 4770 mg/dL ($P = .031$), while median sCD27 levels remained without significant change ($P = .317$). **Conclusion:** sCD27 is a faithful marker of disease burden and is unaffected by the rituximab-induced IgM flare, as well as plasmapheresis in WM. The use of this marker may aid in correctly predicting clinical outcome in patients undergoing treatment with rituximab and/or plasmapheresis in WM.

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Introduction

Waldenström's macroglobulinemia (WM) is an IgM-secreting B-cell lymphoproliferative disorder characterized primarily by bone marrow (BM) infiltration with lymphoplasmacytic cells, and demonstration of an IgM monoclonal gammopathy.¹ This condition is considered to be lymphoplasmacytic lymphoma as defined by the REAL and World Health Organization (WHO) classification systems.² Serum IgM is an important component of disease assessment, which is used to determine therapeutic responses and disease progression in patients with WM.³

Rituximab is an important agent in the treatment of WM, and is

used as monotherapy and in combination with cyclophosphamide, nucleoside analogues, thalidomide and bortezomib.⁴ In many patients with WM, a transient increase of serum IgM might be noted immediately following initiation of rituximab treatment,⁴⁻⁶ and can occur with the use of rituximab alone and in combination therapy.⁷⁻¹¹ The mechanism for the rituximab-induced IgM flare in WM patients remains to be clarified, but does not herald treatment failure and most patients will return to their baseline serum IgM level within a few weeks. However, in some WM patients, an IgM flare lasting > 3 months after rituximab treatment can be observed, despite a marked reduction in the underlying BM disease burden.

Plasmapheresis is also an important therapeutic modality used in the management of many WM patients who are experiencing hyperviscosity-related complications on the basis of high IgM levels, as well as IgM-related autoimmune complications including peripheral neuropathy and hemolytic anemia, as well as cryoglobulinemia.^{12,13} The rapid removal of serum IgM following plasmapheresis often leads to symptomatic improvement in many patients experiencing these IgM-related complications, though

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Table 1 Serum IgM (mg/dL) and sCD27 (U/mL) levels Following a Rituximab-Induced IgM Flare in 8 WM Patients who Ultimately Demonstrated a Response to Treatment

Baseline sIgM	Peak sIgM	Baseline sCD27	Peak sCD27	Baseline BM, %	Peak BM, %	Ultimate Response
3560	7000	171.11	119.52	80	70	MR
3890	6900	299.48	127.46	40	30	SD
3800	5630	174.88	147.3	40	NA	nCR
2880	3430	197.7	160.2	5	NA	MR
3460	3700	166.94	158.41	10	NA	PR
3510	7340	199.68	153.45	30	NA	MR
3520	4910	267.14	171.17	30	NA	PR
1900	2340	222.1	173.29	50	15	PR

Abbreviations: BM = bone marrow; MR = minor response; NA = not available; nCR = near complete response; Peak = at time of peak IgM flare; PR = partial response; sCD27 = soluble CD27; SD = stable disease; sIgM = serum IgM; WM = Waldenström's macroglobulinemia

this complicates further response assessment since the return of steady state IgM levels may take 5-6 weeks.¹³ Moreover, in many patients with WM, ongoing plasmapheresis might be required. As such, novel biomarkers are needed to more accurately assess disease burden in WM patients undergoing rituximab-based therapy, as well as plasmapheresis.

Soluble CD27 (sCD27) is a tumor necrosis factor (TNF) family member, which is secreted by WM cells. In previous studies, we demonstrated that sCD27 could induce the expression of CD40 ligand and A proliferation-inducing ligand (APRIL) on bone marrow mast cells, and through which the growth and survival of WM cells could be supported.¹⁴ Moreover, in this study, sCD27 levels were serially measured in patients whose clinical outcomes (responder, stable disease, or progressive disease) were known, and were found to be highly correlated with serial changes in serum IgM levels in all outcome categories. In as well, sCD27 correlated with the International Prognostic Staging System—untreated WM patients.¹⁵ We therefore performed this study to assess the value of sCD27 as a response marker in patients whose outcomes to therapy were known, and in whom a rituximab-mediated IgM flare was observed, as well as in patients with WM undergoing plasmapheresis.

Patients and Methods

Patient Characteristics

We identified 8 patients with WM who experienced a clinically documented rituximab-mediated IgM flare, for whom serial IgM levels and stored serum samples for sCD27 testing were available. The median age for these patients was 68 years (range, 62-79 years) and the median number of previous therapies was 0 (range, 0-4 therapies). Three of the patients were previously treated, and one of these patients had previously received rituximab in combination with cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP). Therapy leading to the IgM flare consisted of rituximab monotherapy (n = 1), cyclophosphamide/prednisone/rituximab (n = 2), thalidomide/rituximab (n = 4), and lenalidomide/rituximab (n = 2). Seven of these patients achieved a response (1 near complete, 3 partial, 3 minor), and one patient achieved stable disease following therapy. Response assessments were based on consensus criteria using serial changes in serum IgM levels.¹⁶ The

Table 2 Serum IgM (mg/dL) and sCD27 (U/mL) Levels Following Plasmapheresis in 3 WM Patients

Baseline sIgM	Post-PP sIgM	Baseline sCD27	Post-PP sCD27	Baseline BM, %
6380	4770	125.28	123.49	30
6940	3910	116.97	126.17	5
12300	9380	73.04	78.4	40

Abbreviations: BM = bone marrow; PP = plasmapheresis; sCD27 = soluble CD27; sIgM = serum IgM; WM = Waldenström's macroglobulinemia

median baseline IgM was 3515 (range, 1900-3890 mg/dL), and the median BM involvement was 30% (range, 5%-80%). We also identified 3 patients with WM who had undergone plasmapheresis for whom serial IgM levels were available, and stored serum samples for sCD27 were also available. The median age for these patients was 69 years (range, 59-73 years), median baseline serum IgM was 6940 mg/dL (range, 6380-12,300 mg/dL), and median BM involvement was 30% (range, 5%-40%). All 3 patients were treatment naive before plasmapheresis.

ELISA Analysis of WM Patient Serum

All patients provided informed written consent, and the institutional review board approved this study. Serum samples obtained before and after treatment from patients were utilized for these studies. Samples were aliquoted and stored at -80°C, and subsequently quick thawed in a warm bath for determination of sCD27 levels. Previous studies in our laboratory had demonstrated no significant effect of freezing on determination of sCD27 levels (data not shown). To evaluate for sCD27 levels, 100 µL of patient was analyzed by an ELISA kit per manufacturer's instructions (Bender Medsystems, Burlingame CA).

Statistical Analysis

Comparison of pre- and post-treatment parameters was performed using a 2-tailed students t test on Microsoft Excel™ software. A P value ≤ 0.05 was deemed to be significant for the above studies.

Results

Serum IgM and sCD27 Levels Following Rituximab-Induced IgM Flare

Among the 8 patients who experienced a rituximab-mediated IgM flare, IgM levels rose from 3515 to a peak of 5270 mg/dL ($P = .008$). The median time to peak IgM levels post-rituximab treatment for these patients was 51 days (range, 14-177 days). At baseline, the median sCD27 level was 174.1 U/mL, coinciding with the observed baseline serum IgM levels for these 8 patients. Following rituximab treatment and coinciding with peak serum IgM levels, median sCD27 levels declined to 155.9 U/mL ($P = .012$), with a decline observed in all patients (Table 1). In 3 patients, BM biopsies were performed post rituximab treatment which confirmed the presence of an IgM flare, as well as reduction in disease burden predicted by measurement of sCD27 levels (Table 1).

Serum IgM and sCD27 Levels Following Plasmapheresis

Among 3 patients undergoing plasmapheresis, IgM levels declined from 6940 to 4770 mg/dL ($P = .031$) at a median of 2 days (range, 2-29 days). At baseline, the median sCD27 level was 116.9 U/mL, coinciding with the observed baseline serum IgM levels for these 3 patients. Following plasmapheresis, and coinciding with the obtained post-plasmapheresis serum IgM levels (Table 2), no significant change in sCD27 levels occurred (123.9 U/mL; $P = .317$).

Discussion

The assessment of disease burden is often difficult in patients with WM who receive rituximab due to the induction of an IgM flare, and following the removal of serum IgM by plasmapheresis. In such circumstances, serial BM biopsies have been utilized to clarify a patient's underlying disease burden, adding to patient discomfort, delay in response assessment, as well as treatment costs. We therefore evaluated sCD27 as a marker of disease burden in WM patients experiencing a rituximab-related IgM flare, as well as undergoing plasmapheresis. sCD27 is a TNF family member secreted by WM cells through proteolytic cleavage, which we previously showed correlated strongly with disease progression and response. The results of the present study demonstrate that sCD27 is a faithful marker of disease burden in patients experiencing a rituximab-related IgM flare, and correctly predicted response

outcome. In as well, sCD27 levels remained unaffected by plasmapheresis in WM patients. The results of this study support the broader investigation of sCD27 as an adjunct in the clinical management of patients undergoing treatment with rituximab and/or plasmapheresis. Further validation of the use of sCD27 in other WM disease related circumstances where IgM levels are potentially unreliable such as cryoglobulinemia, or bortezomib-related paraprotein suppression might lead to additional roles for the use of sCD27 in the management of patients with WM.

References

- Owen RG, Treon SP, Al-Katib A, et al. Clinicopathological definition of Waldenström's macroglobulinemia: Consensus Panel Recommendations from the Second International Workshop on Waldenström's macroglobulinemia. *Semin Oncology* 2003; 30:110-5.
- Harris NL, Jaffe ES, Diebold J, et al. World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues: report of the Clinical Advisory Committee meeting- Airlie House, Virginia, November 1997. *J Clin Oncol* 1999; 17:3835-49.
- Kimby E, Treon SP, Anagnostopoulos A, et al. Update on recommendations for assessing response from the Third International Workshop on Waldenström's Macroglobulinemia. *Clin Lymphoma Myeloma* 2006; 6:380-3.
- Dimopoulos MA, Zervas C, Zomas A, et al. Treatment of Waldenström's macroglobulinemia with rituximab. *J Clin Oncol* 2002; 20:2327-33.
- Treon SP, Branagan AR, Hunter Z, et al. Paradoxical increases in serum IgM and viscosity levels following rituximab in Waldenström's macroglobulinemia. *Ann Oncol* 2004; 15:1481-3.
- Ghobrial IM, Fonseca R, Greipp PR, et al. Initial immunoglobulin M "flare" after rituximab therapy in patients with Waldenström Macroglobulinemia: An Eastern Cooperative Oncology Group Study. *Cancer* 2004; 101:2593-8.
- Nichols GL, Savage DG. Timing of rituximab/fludarabine in Waldenström's macroglobulinemia may avert hyperviscosity. *Blood* 2004; 104:237b.
- Treon SP, Soumerai JD, Branagan AR, et al. Thalidomide and Rituximab in Waldenström's macroglobulinemia. *Blood* 2008; 112:4452-7.
- Treon SP, Soumerai JD, Branagan AR, et al. Lenalidomide and Rituximab in Waldenström's macroglobulinemia. *Clin Cancer Res* 2008; 15:355-60.
- Treon SP, Ioakimidis L, Soumerai JD, et al. Primary therapy of Waldenström's macroglobulinemia with bortezomib, dexamethasone and rituximab: Results of WMCTG Clinical Trial 05-180. *J Clin Oncol* 2009; [In press].
- Ioakimidis L, Patterson C, Soumerai J, et al. Comparative outcomes following CP-R, CVP-R and CHOP-R in patients with Waldenström's macroglobulinemia. *Clin Lymphoma Myeloma* 2009; [In press].
- Gertz M, Anagnostopoulos A, Anderson KC, et al. Treatment recommendations in Waldenström's macroglobulinemia: consensus panel recommendations from the Second International Workshop on Waldenström's Macroglobulinemia. *Semin Oncol* 2003; 30:121-6.
- Menke MN, Treon SP. Hyperviscosity Syndrome. In: *Clinical Malignant Hematology*. Sekeres, Kalaycio, Bolwell eds. New York: McGraw Hill Publishing, 2007.
- Ho AW, Hatjiharissi E, Branagan AR, et al. CD27-CD70 interactions in the pathogenesis of Waldenström's Macroglobulinemia. *Blood* 2008; 112:4683-9.
- Hatjiharissi E, Ciccarelli BT, Xu L, et al. Soluble CD27 levels are correlated with disease stage and response to treatment in Waldenström's macroglobulinemia (WM). *Ann Oncology* 2008; 19:177 (Abstract 314).
- Kimby E, Treon SP, Anagnostopoulos A, et al. Update on recommendations for assessing response from the Third International Workshop on Waldenström's Macroglobulinemia. *Clin Lymphoma Myeloma* 2006; 6: 380-3.