B-lymphocyte stimulator: can we consider it a marker for severity of hepatitis C virus-induced B-cell non-Hodgkin lymphoma?

Yousryeia A. Ahmad\textsuperscript{a}, Ola Afifi\textsuperscript{b}, Safinaz Hussein\textsuperscript{a}, Rania Hafez\textsuperscript{a}, Eman Salaheldin\textsuperscript{b}

\textsuperscript{a}Department of Internal Medicine, Clinical Hematology Unit, \textsuperscript{b}Department of Clinical Pathology, Assiut University, Assiut, Egypt

Correspondence to Rania Hafez, MD, Department of Internal Medicine, Assiut University, Assiut - 71516, Egypt
Tel: +20 100 001 9198; fax: +2068233327; e-mail: raniahafez@ymail.com

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Objective
B-lymphocyte stimulator (BLyS) is a member of tumor necrosis factor family. BLyS is essential for the survival of normal and malignant B lymphocyte. Hepatitis C virus (HCV) infection likely represents the early event leading to BLyS upregulation. The aim of this study was to determine the relation between serum BLyS levels and the severity of HCV-related B-cell non-Hodgkin lymphoma (B-NHL).

Patients and methods
Seventy-eight B-NHL patients both HCV-positive and HCV-negative and 20 patients with HCV infection without lymphoproliferative disorders, in addition to 20 age-matched and sex-matched controls, were included in the study. PCR for HCV, evaluation of levels of soluble BLyS protein in blood, bone marrow aspirate and biopsy with flow cytometry, and lymph node biopsy with immunophenotyping for CD5, CD23, CD10, CD20, and cyclinD1 were performed.

Results
The serum BLyS levels were significantly higher in B-NHL patients and HCV patients without lymphoproliferative disorders compared with the control group. The serum BLyS levels were statistically significantly higher in aggressive lymphoma patients with HCV-positive infection compared with aggressive lymphoma patients with HCV-negative infection, but there was no statistically significant difference between BLyS levels in indolent B-NHL patients with or without HCV infections. Moreover, there was no statistically significant difference between BLyS levels in aggressive and indolent lymphoma patients with HCV-positive infection.

Conclusion
BLyS levels are increased in HCV-induced B-NHL but it cannot be considered as a marker for severity of the disease (indolent or aggressive). More studies are needed.

Keywords:
B cell, B-cell-activating factor, B-cell non-Hodgkin lymphoma, B-lymphocyte stimulator, hepatitis C virus, lymphoma

Introduction
Egypt has the highest hepatitis C virus (HCV) prevalence in the world with an estimated prevalence of 14.7\% among those between 15 and 59 years of age [1–4].

HCV carriers are prone to development of chronic hepatitis, cirrhosis, and hepatocellular carcinoma [5]. Moreover, long-standing infection with HCV can lead to the development of extrahepatic manifestations of the disease, such as mixed cryoglobulinemia and other immune complex-mediated disorders due to B-cell proliferation that may finally evolve into overt B-cell non-Hodgkin’s lymphoma (B-NHL) [6,7].

Farawela et al. [8] noticed a significantly higher prevalence of HCV infection among newly diagnosed NHL patients before therapy relative to controls, and this conferred a 14-fold increased NHL risk with HCV infection.

It has been suggested that HCV plays an important role in the pathogenesis of B-NHL as many non-Hodgkin’s lymphoma (NHL) subtypes are associated with HCV infection, such as diffuse large B-cell lymphoma (DLBCL), mantle cell lymphoma (MCL), chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), and lymphoplasmacytic lymphoma [9].
It has been postulated that HCV is responsible for B-lymphocyte stimulator (BLyS) upregulation, which acts as a stimulus for B-cell autoimmunity and lymphoproliferation in infected patients [10].

BLyS, also called B-cell-activating factor, is a member of tumor necrosis factor family, which causes B-cell growth stimulation [11]. It is expressed in a variety of cell types, such as peripheral blood leukocytes and stromal cells of the spleen and lymph nodes [12,13]. It is an important factor affecting B-cell proliferation, survival, maturation, and antibody production [11].

Abnormal production of BLyS in NHL by malignant cells themselves or by surrounding microenvironment can protect these cells from programmed spontaneous death and promote their growth and survival [14].

Thus, the aim of this study was to estimate BLyS levels in patients with HCV-induced B-NHL and correlate it with the severity of the disease as aggressive or indolent lymphoma.

**Patients and methods**

This prospective nonrandomized study was conducted at the Clinical Hematology Unit, Internal Medicine Department, Faculty of Medicine, and South Egypt Cancer Institute, Assiut University, between January 2014 and December 2016. This study included 78 patients with B-NHL (group I), both HCV-positive (n=25, 32.1%) and HCV-negative (n=53, 67.9%). Their ages ranged from 17 to 75 years with a mean of 50.56±1.62 years. According to REAL classification 2008, from the total 78 patients of group I, 33 (42.3%) patients were diagnosed with indolent lymphoma (CLL/SLL), whereas the remaining 45 (57.7%) patients were diagnosed with aggressive lymphoma (42 patients with DLBCL and three patients with MCL).

The study included 20 patients (group II) with HCV infection without lymphoproliferative disorders, in addition to 20 age-matched and sex-matched healthy individuals as a control group.

From the total 78 patients (group I) with B-NHL, 33 (42.3%) patients were diagnosed with CLL/SLL, 42 (53.8%) patients with DLBCL, and the remaining three (3.8%) patients with MCL. From the 33 patients with CLL/SLL, nine patients were positive for HCV infection, whereas the remaining 24 patients were negative, and of 42 patients with DLBCL 14 patients had positive HCV infection, whereas 28 patients were negative. Two patients of those diagnosed with MCL had positive HCV infection, whereas one patient was negative for HCV infection.

The serum BLyS levels were significantly increased in group I (median: 6.8 ng/ml, mean±SE: 11.68±1.78, range: 1.1–64 ng/ml) and group II (median: 7 ng/ml, mean±SE: 6.49±0.41, range: 3.4–9 ng/ml) when compared with the control group (median: 3.2 ng/ml, mean±SE: 3.13±0.25, range: 0.8–5 ng/ml) with P-value less than 0.01 for both comparisons (Tables 1 and 2).

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There were no statistically significant differences in serum BLyS levels in aggressive lymphoma patients (DLBCL and MCL) as the median of BLyS levels was
7.5 ng/ml (mean±SE: 11.24±2.63, range: 1.1–60 ng/ml) when compared with the serum BLyS levels in indolent lymphoma patients (CLL/SLL) as the median of the serum BLyS levels was 5.8 ng/ml (mean±SE: 11.15±2.44, range: 1.2–64 ng/ml) with P-value equal to 0.567 (Table 3).

The serum BLyS levels were statistically significantly higher in aggressive lymphoma patients with HCV-positive infection (median: 7.8 ng/ml, mean±SE: 15.21±5.56, range: 3.6–64 ng/ml) when compared with aggressive lymphoma patients with HCV-negative infection (median: 5.5 ng/ml, mean±SE: 9.66±2.77, range: 1.2–62) with P-value equal to 0.041. However, there were no statistically significant differences in indolent lymphoma patients with HCV-positive infection (median: 6.8 ng/ml, mean±SE: 11.09±5.19, range: 2.3–52 ng/ml) when compared with indolent lymphoma patients with HCV-negative infection (median: 7.7 ng/ml, mean±SE: 12.81±3.36, range: 1.1–60 ng/ml) with P-value equal to 0.671 (Table 4).

However, there was no statistically significant difference in BLyS levels between indolent and aggressive lymphoma patients with positive HCV (Table 5).

### Table 1 The relation between serum B-lymphocyte stimulator levels in non-Hodgkin lymphoma patients and control group

| Mean±SE | Median | Range | P-value |
|---------|--------|-------|---------|
| NHL patients | 11.68±1.78 | 6.8 | 1.1–64.0 | <0.01 |
| Control | 3.13±0.25 | 3.2 | 0.8–5.0 | |

NHL, non-Hodgkin lymphoma.

### Table 2 Relation between serum B-lymphocyte stimulator levels in hepatitis C virus infected patients and control group

| BLyS | HCV (n=20) | Control (n=20) | P-value |
|------|------------|----------------|---------|
| Mean±SE | 6.49±0.41 | 3.13±0.25 | <0.01 |
| Median | 7.0 (3.4–9.0) | 3.2 (0.8–5.0) | |

BLyS, B-lymphocyte stimulator; HCV, hepatitis C virus.

### Table 3 Serum B-lymphocyte stimulator levels in indolent and aggressive lymphoma patients

| B-lymphocyte NHL patients | Mean±SE | Median | Range | P-value |
|---------------------------|---------|--------|-------|---------|
| Aggressive lymphoma patients (DLBCL and MCL) | 11.24±2.63 | 7.5 | 1.1–60.0 | 0.567 |
| Indolent lymphoma patients (CLL/SLL) | 11.15±2.44 | 5.8 | 1.2–64.0 | |

BLyS, B-lymphocyte stimulator; CLL/SLL, chronic lymphocytic leukemia/small lymphocytic lymphoma; DLBCL, diffuse large B-cell lymphoma; MCL, mantle cell lymphoma.

### Table 4 The relation between serum B-lymphocyte stimulator levels in indolent lymphoma patients and aggressive lymphoma patients (positive and negative hepatitis C virus infection)

| B-cell NHL patients | BLyS levels (ng/ml) | P-value |
|---------------------|---------------------|---------|
| Indolent lymphoma patients (n=9 CLL/SLL) with HCV-positive infection | Median (range) | Mean±SE |
| 6.8 (2.3–52) | 11.09±5.19 | 0.671 |
| Indolent lymphoma patients (n=24 CLL/SLL) with HCV-negative infection | 7.7 (1.1–60) | 12.81±3.36 |
| Aggressive lymphoma patients (n=14 DLBCL+2 MCL) with HCV-positive infection | 7.8 (3.6–64) | 15.21±5.58 |
| Aggressive lymphoma patients (n=28 DLBCL+1 MCL) with HCV-negative infection | 5.5 (1.2–62) | 9.66±2.77 |

BLyS, B-lymphocyte stimulator; CLL/SLL, chronic lymphocytic leukemia/small lymphocytic lymphoma; DLBCL, diffuse large B-cell lymphoma; HCV, hepatitis C virus; MCL, mantle cell lymphoma; NHL, non-Hodgkin lymphoma.

### Table 5 The relation between serum B-lymphocyte stimulator levels in indolent and aggressive lymphoma patients with positive hepatitis C virus infection

| B-cell NHL patients | BLyS levels (ng/ml) | P-value |
|---------------------|---------------------|---------|
| Indolent lymphoma patients (n=9 CLL/SLL) with HCV-positive infection | Median (range) | Mean±SE |
| 6.8 (2.3–52) | 11.09±5.19 | 0.541 |
| Aggressive lymphoma patients (n=14 DLBCL+2 MCL) with HCV-positive infection | 7.8 (3.6–64) | 15.21±5.56 |

BLyS, B-lymphocyte stimulator; CLL/SLL, chronic lymphocytic leukemia/small lymphocytic lymphoma; DLBCL, diffuse large B-cell lymphoma; HCV, hepatitis C virus; MCL, mantle cell lymphoma; NHL, non-Hodgkin lymphoma.

### Discussion

HCV infection is the most common chronic viral infection worldwide affecting about 180 million people, which accounts for 3% of the world population and it is endemic in Egypt [15].

BLyS, a tumor necrosis factor family member, is a very important factor in B-cell survival and maturation [16]. It has been suggested that HCV infection may present an early stimulator to BLyS upregulation, leading to progression into B-NHL [10].

In the current study serum BLyS levels were significantly higher in patients with HCV infection...
without lymphoproliferative disorders compared with controls ($P<0.01$). This is in agreement with previous reports such as that by Fabris et al. [10], who found that 10/33 (30.3%) of patients with HCV infection have high serum BLYS levels ($P=0.0026$) compared with controls, and Ito et al. [17], who reported elevated serum BLYS levels in patients with HCV than in controls ($P<0.001$) and they added that HCV infection may induce the release of BLYS.

In our results, serum BLYS levels were significantly elevated in patients with B-NHL when compared with the control group ($P<0.01$). Our results are in agreement with Yang et al. [18], who found that serum BLYS levels were significantly elevated in patients with most NHL compared with those in healthy donors, and it correlated with aggressive disease and a poor response to therapy. Haiat et al. [19] also detected that the levels of soluble BLYS in patients with different NHL subtypes were significantly elevated when compared with those in healthy controls.

In our results we detected increased BLYS levels in 23 of 33 CLL/SLL, 37 of 42 of DLBCL, and three of three MCL, and thus BLYS levels were detected in all histological subtypes of B-NHL. Our results correlated with that of Novak et al. [20], who detected BLYS in five of five MCL, five of five DLBCL, three of four B-cell CLL/SLL and one of five follicular lymphoma; BLYS was detected in all NHL specimens studied with variable levels of BLYS detected within the histological subtypes. However, in their study they found that there were no statistically significant differences between serum BLYS level among the healthy control group, indolent lymphoma patients, and aggressive lymphoma. This is in contrast with our results as we detected significantly elevated levels in patients with B-NHL when compared with the control group ($P<0.01$) and they believed that this was likely due to the reagents used in their ELISA.

In this study serum BLYS levels were higher in aggressive lymphoma patients with HCV-positive infection when compared with aggressive lymphoma patients with HCV-negative infection ($P=0.041$). However, there were no statistically significant differences in indolent lymphoma patients with HCV-positive infection when compared with indolent lymphoma patients with HCV-negative infection ($P=0.671$).

## Conclusion

BLYS serum levels are elevated in NHL subtypes suggesting that excessive production of BLYS occurs in these patients. HCV infection likely represents the early event leading to BLYS upregulation. BLYS can be considered as a marker for HCV-induced B-NHL. However, it does not indicate severity of the disease as there was no significant difference between indolent and aggressive lymphomas with HCV-positive infection.

## Recommendations

More studies are needed with a larger number of patients and correlation of BLYS levels with outcome and survival of patients after therapy.

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## Conflicts of interest
There are no conflicts of interest.

## References

1. Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. Lancet Infect Dis 2005; 5:558–567.
2. Alter MJ. Epidemiology of hepatitis C virus infection. World J Gastroenterol 2007; 13:2436–2441.
3. Lavanchy D. Evolving epidemiology of hepatitis C virus. Clin Microbiol Infect 2011; 17:107–115.
4. Mohamoud YA, Muntaz GR, Riome S, Miller D, Abu-Raddad LJ. The epidemiology of hepatitis C virus in Egypt: a systematic review and data synthesis. BMC Infect Dis 2013; 13:288.
5. Afzal NH. The natural history of hepatitis C. Semin Liver Dis 2004; 24 (Suppl 2):3–8.
6. Zuckerman E, Zuckerman T, Levine AM, Douer D, Gutekunst K, Mizokami M, et al. Hepatitis C virus infection in patients with B-cell non-Hodgkin lymphoma. Ann Intern Med 1997; 127:423–428.
7. Ito M, Murakami K, Suzuki T, Mochida K, Suzuki M, Ikeuchi K, et al. Enhanced expression of lymphomagenesis-related genes in peripheral blood B cells of chronic hepatitis C patients. Clin Immunol 2010; 135:459–465.
8. Farawela H, Khorsheed M, Shaheen I, Gouda H, Nasef A, Abulata N, et al. The association between hepatitis C virus infection, genetic polymorphisms of oxidative stress genes and B-cell non-Hodgkin’s lymphoma risk in Egypt. Infect Genet Evol 2012; 12:1189–1194.
9. Viswanatha DS, Dogan A. Hepatitis C virus and lymphoma. J Clin Pathol 2007; 60:1378–1383.
10. Fabris M, Quartuccio L, Sacco S, De Marchi G, Pozzato G, Mazzaro C, et al. B-lymphocyte stimulator (BLYS) up-regulation in mixed cryoglobulinaemia syndrome and hepatitis-C virus infection. Rheumatology (Oxford) 2007; 46:37–43.
11. Uchikoshi M, Takayoshi I, Shimozuma Y, Inokuchi M, Morikawa K, Nozawa H, Imawari M. Serum B cell activating factor (BAFF/BLYS) as an immunological marker for lymphoproliferative disorders associated with chronic hepatitis C in Japanese patients. Showa Uni J Med Sci 2009; 21:55–65.
12. Dejardin E, Drouin NM, Delhase M, Haas E, Cao Y, Makris C, et al. The lymphoxygenin-beta receptor induces different patterns of gene expression via two NF-kappaB pathways. Immunity 2002; 17:525–535.
13. Lesley R, Xu Y, Kalled SL, Hess DM, Schwab SR, Shu HB, Cyster JG. Reduced competitiveness of autoantigen-engaged B cells due to increased dependence on BAFF. Immunity 2004; 20:441–453.
14. Lied GA, Berstad A. Functional and clinical aspects of the B-cell-activating factor (BAFF): a narrative review. Scand J Immunol 2011; 73:1–7.
15. El-Hefni AM, Elghohary TA, Kott A, Abu-Taleb FM. Efficacy of ribavirin to prevent hepatitis reactivation in hepatitis C virus-infected patients treated for non-Hodgkin lymphoma. Afro-Egyptian J Infect Endemic Dis 2013; 2:14–21.
16. Cohen S, Shachar I. Midkine as a regulator of B cell survival in health and disease. Br J Pharmacol 2014; 171:888–895.
17 Ito M, Mizoroki F, Takai K, Yamaguchi K, Mizuochi T. Functional phenotypes and gene expression profiles of peripheral blood mononuclear cells in chronic hepatitis C patients who developed non-Hodgkin’s B-cell lymphoma. Biochem Biophys Res Commun 2009; 390:269–272.

18 Yang S, Li JY, Xu W. Role of BAFF/BAFF-R axis in B-cell non-Hodgkin lymphoma. Crit Rev Oncol Hematol 2014; 91:113–122.

19 Haiat S, Billard C, Quiney C, Ajchenbaum-Cymbalista F, Kolb JP. Role of BAFF and APRIL in human B-cell chronic lymphocytic leukaemia. Immunology 2006; 118:281–292.

20 Novak AJ, Grote DM, Stenson M, Ziesmer SC, Witzig TE, Habermann TM, et al. Expression of BLyS and its receptors in B-cell non-Hodgkin lymphoma: correlation with disease activity and patient outcome. Blood 2004; 104:2247–2253.