Can Gilbert’s syndrome mitigate chronic lymphocytic leukemia?

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ARTICLE INFO

Keywords:
Leukemia
Lymphocytic
Gilbert’s
Syndrome

ABSTRACT

The study links two intriguing observations about chronic lymphocytic leukemia and Gilbert’s syndrome – the oxidative stress affecting the former, and the anti-oxidative effects of the latter. Our pilot study showed that compared to the general CLL population, the GS/CLL cohort less commonly required therapy and demonstrated a reduced CLL-related mortality. Our findings prompt a speculation that elevated bilirubin in GS could hypothetically attenuate the oxidative stress thereby exerting a safeguarding effect on leukemia pathogenesis. We believe that this hypothesis-generating study could open new avenues for exploring oxidative stress as a potential pathogenetic and, hypothetically, therapeutic target for mitigating CLL.

Gilbert’s syndrome (GS) is the most common inherited disorder of bilirubin glucuronidation. The GS results from a defect in the gene encoding the enzyme uridine diphosphate (UDP)-glucuronosyltransferase 1A1 (UGT1A1), which is responsible for conjugation of bilirubin with glucuronic acid. Affected individuals exhibit isolated, mild elevation in unconjugated bilirubin (usually caused by stress such as fasting, febrile illness, or physical exertion) without apparent liver, biliary or red blood cell injury. Besides being innocuous, GS has been associated with many health benefits such as greatly reduced risk for cardiovascular diseases, diabetes mellitus, respiratory diseases as well as all-cause mortality [1–3]. The positive health effects have been largely attributed to bilirubin’s antioxidant properties [4]. On the other hand, UGT1A1 affects metabolism of many drugs, hormones and toxins leading to pharmacogenetic and potential health risks [3].

The impact of GS on the course of cancers [3] and hematological malignancies in particular remains uncertain. The higher incidence of GS has been reported in childhood acute leukemia (14–20%) [5–7], but no differences in outcome were found in patients with GS [7]. GS has also been associated with excessive toxicity of anticancer drugs in childhood acute lymphoblastic leukemia [8], but improved outcomes in adult patients with Hodgkin lymphoma [9]. To date, no studies have addressed the incidence or putative pathogenetic effect of GS in CLL, despite the high prevalence of both conditions worldwide. We hypothesized that, because the oxidative stress has been implicated in pathogenesis of CLL correlating with greater likelihood of cytogenetic abnormalities including 17p deletion, more aggressive course, and poor response to treatment [10,11], the elevated bilirubin in GS could hypothetically mitigate severity of CLL.

Our pilot study explored the incidence of GS among the CLL patients in Israel. Additionally, relevant clinical, laboratory and epidemiological characteristics including male-to-female ratio, Ashkenazi-to-Sephardic Jew ratio, CLL-related deaths, and overall survival among the GS/CLL patients were compared to the general CLL population. Between August 1990 and June 2020, 778 patients with CLL and bilirubin measurements at diagnosis were retrospectively identified from the Kaplan Medical Center database (Fig. 1). GS diagnosis required at least two unconjugated bilirubin readings [12] greater than 0.7 mg/dL (reference range for total bilirubin of 0.3–1.2 mg/dL and for conjugated bilirubin of 0–0.5 mg/dL), normal serum transaminases and negative markers of the biliary and red blood cell damage. Of 778 patients, 37 exhibited unconjugated hyperbilirubinemia (UB). Twelve of these patients were excluded because of the evidence of liver disease or conditions that could have affected bilirubin results: hepatitis B (3), idiopathic hepatitis (3), cryptogenic liver cirrhosis (2), HCV hepatitis (1) and autoimmune hemolytic anemia (3). Twenty-five patients were diagnosed as having GS. Their relevant clinical, laboratory and epidemiological characteristics are presented in Table 1.

Our study covered a period of 30 years and showed the rate of GS among CLL patients (GS/CLL) of 3.21% (CI 95%, 1.97–4.45) (Table 1). Although the incidence of GS among the general Israeli population has not been systematically studied to our knowledge, the incidence of GS among CLL was the lowest compared to the general population across most geographical regions [13].

Recognizing the limitations of the small sample size, we correlated

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https://doi.org/10.1016/j.lrr.2022.100322
Received 13 October 2021; Received in revised form 21 April 2022; Accepted 4 May 2022
Available online 6 May 2022
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the data of GS/CLL patients with the two control groups - our own CLL patients without GS and the historical CLL cases. Table 1 depicts the sex, ethnic background, Binet stage distribution, beta 2 microglobulin and immunoglobulin levels, cytogenetics by FISH, survival and treatment received by the GS/CLL cohort, our general CLL group, and the patients reported by the Israeli CLL Study Group [14]. The table demonstrates that, while some parameters such as age and CLL stage distribution in GS/CLL group matched the controls, several others exhibited differences. Thus, concerning the patients’ sex distribution, the male predominance in the GS/CLL cohort was noticeably higher than in the two control groups: 76% vs 56.6% and 57.6%, correspondingly. The cause of such a difference is not entirely clear. It is noteworthy that, across several countries, the incidence of GS in males has been shown to be twice as high as in females [2,13]. According to our analysis (not shown), this sex difference could have accounted for higher prevalence of males over females in our cohort.

Aside from the recognized sex differences, GS prevalence varies among ethnically different geographic regions. For example, individuals with Eastern Asian ancestry appear to have the lowest bilirubin concentrations (prevalence of GS is about 2%), whereas individuals originating from India, Southern Asia and the Middle East demonstrate increased rates of GS approximating 20% [13] with the highest rate recorded in Africa and among African Americans. Given the ethnic variations in GS prevalence, we compared the incidence of GS between the two main ethnic divisions, Ashkenazi and Sephardic Jews. Surprisingly, the proportion of Ashkenazi Jews was much lower among the GS/CLL patients than in general CLL population in Israel: 48% vs 62.4% and 68.6% (Table 1) [14,15]. Although no comparative studies on the incidence of GS in Ashkenazi Jews vs Sephardic Jews are available to our knowledge, it is interesting to speculate that the differing results could stem from conceivably geographically different prevalence of GS in areas where the two groups historically resided. The median survival was not reached in the GS/CLL cohort, but it was not statistically different from the two control groups (9.0 years and 10.9 years) (p = 0.87). However, a proportion of CLL-related deaths was smaller in the GS/CLL cohort (36.3%) compared to the both control groups (50.5% and 53.7%) (Table 1), and less GS/CLL patients required treatment: 28% vs 41.7% and 44.8%, correspondingly. The smaller proportion of CLL-related deaths was not affected by the follow-up time because the GS rate among CLL patients was similar in the three consecutive decades: 3.09% (6 of 194) in 1990–2000, 3.17% (9 from 284) in 2001–2010, and 3.0% (10 of 300) in 2011–2020. Furthermore, the median year of CLL diagnosis among our CLL/GS patients was comparable with the median year of GS diagnosis, 2008 vs 2006, correspondingly.

One of the study limitations was its retrospective nature. Further, despite the large CLL patient population studied, the number of the patients with GS was relatively small, which disallowed unequivocal assertions regarding the GS effect on CLL course and survival. Besides, the pathogenetic factors independent of oxidative stress could have confounded our results. The incidence of the GS/CLL cases could have been underestimated because, in the absence of stress, many individuals exhibit bilirubin levels below the upper limit of normal, but this is true across many other studies. Moreover, we used a traditional diagnostic criterion that requires at least two abnormal UB readings [12]. One of the strengths of our study was that it was carried out among the CLL patients over the course of the same period that matched the larger CLL population that had been rigorously analyzed previously [14,15] permitting an in-depth comparative analysis.

In summary, we present the first study on putative impact of GS on CLL. As compared to the general CLL population, the GS/CLL cohort less commonly required therapy and demonstrated a reduced CLL-related

![Fig. 1. Flowchart of the patients.](image-url)
safeguarding effect on leukemia pathogenesis. We believe that this

Table 1
Clinical and epidemiological data on patients with CLL and CLL with Gilbert syndrome.

|                        | Our CLL patients without GSN | Historical controls1 N = 1325 | GS/CLL N = 25 |
|------------------------|------------------------------|-------------------------------|---------------|
| Age, years (mean)      | 34.98 (69.5)                 | 32.94 (69.0)                 | 42.87 (68.9)  |
| Sex:                   |                              |                               |               |
| Males                  | 428 (56.6%)                  | 764 (57.6%)                  | 19 (76%)      |
| Females                | 325 (43.4%)                  | 552 (41.9%)                  | 6 (24%)       |
| Ethnic origin:         |                              |                               |               |
| Ashkenazi (%)          | 470 (62.4%)                  | 909 (68.6%)                  | 12 (48%)      |
| Sephardi (%)           | 277 (36.8%)                  | 384 (28.9%)                  | 13 (52%)      |
| Others (%)             | 6 (0.8%)                     | 32 (2.4%)                    | -             |
| Binet stage (%):       |                              |                               |               |
| A                      | 599 (79.5%)                  | 934 (70.7%)                  | 20 (80%)      |
| B                      | 110 (14.6%)                  | 250 (18.9%)                  | 1 (4%)        |
| C                      | 44 (5.8%)                    | 134 (10.2%)                  | 4 (16%)       |
| Missing                | -                            | 16                            | -             |
| Immunoglobulins, tested/reduced |
| Reference range:       |                              |                               |               |
| IgG, 700-1600 mg/dL     | 507/81 (15.9%)               | (14.1%)                      | 15/1          |
| IgA, 70-499 mg/dL       | 530/164 (36.8%)              | n/a                           | 6 (5.7%)      |
| IgM, 40-230 mg/dL       | (30.9%)                      | n/a                           | 15/5          |
| Serum beta-2 microglobulin, tested/ elevated |
| Reference range:       | 0.1-90 mg/L                  |                               |               |
| Cytogenetics by FISH studies |
| Tested/abnormal         | 239/187 (79.2%)              | 125/74 (59.2%)               | 7/4 (57%)     |
| Treated patients with appropriate follow-up (%) | 297/712 (41.7%) | 567 (44.8%) | 7 (28%) |
| Median survival (years): | 9.0 years                    | Not                           | 148           |
| Alive (patients)        | 343                          | 622                           | reached       |
| Dead                   | 406                          | 685                           | 148           |
| Unknown                | 4                             | 9                             | 1             |
| Cause of death          |                              |                               |               |
| CLL (including infections) | 205/406 (63.6%)            | 368 (53.7%)                  | 4 (36.3%)     |
| Other                  | 196/406 (63.6%)              | 293 (42.8%)                  | 7             |
| Unknown                | (48.3%)                      | 24 (3.5%)                    | (63.6%)       |
| Mortality              |                              | 5/406 (1.2%)                 | -             |

1Historical controls were borrowed from the data of the Israeli CLL Study Group [13].

2IgM was reduced in one patient (38 mg/dL).

3IgA was reduced in five patients (16, 17, 17, 30, 34 mg/dL).

4Serum beta-2 microglobulin levels were elevated in eight patients (2.0; 2.0; 2.1; 2.1; 2.2; 2.9; 5.2; 5.7 mg/L).

5Abnormal FISH studies included: 12+ in one patient, 13q- in two patients, 11q/13q- in one patient.

6One patient each was treated with rituximab/cyclophosphamide/vinricistine/prednisone (R-CVP), CVP, chlorambucil/obinutuzumab, venetoclax, fludarabine/cyclophosphamide/rituximab and two patients with CP.

7Data were calculated among the assessable historical controls.

8Surviving patients are alive from 30 to 284 months.

9CLL-related deaths: survival time, 62, 90, 108, and 113 months.

10Non-CLL related deaths: renal failure (two) including one drug-induced, Kaposi sarcoma, myelodysplastic syndrome, cardiac event (two), and stroke.

oxidative stress as a potential pathogenetic and, hypothetically, therapeuetic target for mitigating CLL.

Funding statement
none

Data sharing
The original data is available upon request.

Authorship contributions
LLY proposed the idea and design of the study, analyzed the data, wrote the paper and approved submitted version. LS collected the data, analyzed the data, reviewed, contributed to, and approved the manuscript.

Declaration of Conflicts of Interest
None to report

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