Al-Rafidain J Med Sci. 2026;10(2):73-78.
DOI: https://doi.org/10.54133/ajms.v10i2.2861

AJMS

Research Article

Online ISSN (3219-2789)

Dynamics of Soluble HLA-G and the 14-bp Insertion/Deletion Polymorphism as Predictors of Therapeutic Response in Acute Myeloid Leukemia

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Received: 25 February 2026; Revised: 2 April 2026; Accepted: 6 April 2026

Abstract

Background: Acute myeloid leukemia (AML) exploits immune escape mechanisms such as human leukocyte antigen-G (HLA-G), a non-classical HLA-I molecule involved in immune checkpoints and associated with the 14-bp insertion/deletion (Ins/Del) polymorphism for cancer prognosis. Prospective data regarding the dynamics of these parameters and their relation to therapy outcomes in AML patients remains limited. ***Objectives***: Determine the relationship between the serum level of soluble human leukocyte antigen-G (sHLA-G) and the 14-bp deletion/insertion (Ins/Del) polymorphism of the sHLA-G gene in AML patients before and after chemotherapy. ***Methods***: A longitudinal study with a prospective follow-up was executed. Forty-six adult patients diagnosed with AML were recruited. ELISA was used to measure serum levels of sHLA-G at diagnosis (T0) and six months after induction chemotherapy (T1). PCR genotyping was done on the HLA-G 14-bp polymorphism. This study focuses on the interaction of sHLA-G dynamics and genotype with the outcomes of clinical response, which could be Complete Remission (CR) or Non-Remission (NR). ***Results***: sHLA-G levels reduced markedly after induction chemotherapy (28.5±15.3ng/mL at T0 *vs*. 7.6±6.1ng/mL at T1, $p<0.001$). Baseline sHLA-G values greater than 30ng/mL were significant predictors of non-response to treatment, demonstrating 82% sensitivity and 70% specificity. However, there were no significant variations in the distribution of HLA-G 14-bp genotypes between responders and non-responders ($p=0.45$). ***Conclusions***: sHLA-G is a sensitive and dynamic biomarker of leukemic load; high baseline levels are significantly associated with non-response to induction therapy. In contrast, the HLA-G 14-bp polymorphism was not identified as an independent predictor of therapeutic response in this cohort.

Keywords: Acute myeloid leukemia; Chemotherapy response; Leukemic burden; Soluble HLA-G.

ديناميكيات HLA-G القابل للذوبان وتعدد الاشكال الناتج عن ادراج/حذف 14 زوجا قاعديا كمؤشرات للاستجابة العلاجية في ابيضاض الدم النخاعي الحاد

الخلاصة

الخلفية: يستغل ابيضاض الدم النخاعي الحاد آليات التهرب المناعي، مثل مستضد الكريات البيضاء البشرية (HLA-G)، وهو جزئ غير كلاسيكي يشارك في نقاط التفتيش المناعية. وقد رُبط كل من HLA-Gالذائب وتعددالأشكال الناتج عن الإدراج/الحذف14زوجًا قاعديًا بتوقعات سيرالمرض؛ ولا تزال البيانات المستقبلية المتعلقة بديناميكية هذه المؤشرات وعلاقتها بنتائج العلاج لدى مرضى ابيضاض الدم النخاعي الحاد محدودة. **الأهداف**: تحديد مستوى (sHLA-G) في مصل مرضى ابيضاض الدم النخاعي الحاد قبل وبعد العلاج الكيميائي والكشف عن تعدد الأشكال الناتج عن الحذف/الإدراج 14زوجًا قاعديًا في جينHLA-G و إيجاد علاقة بين هذه المؤشرات والنتائج السريرية للمرضى. **الطرائق**: أُجريت دراسة طولية مع متابعة مستقبلية. تم تحديد 46 مريضًا بالغًا بعد تشخيص إصابتهم بابيضاض الدم النخاعي الحاد.استُخدم اختبارELISAلقياس مستويات sHLA-G في مصل الدم عند التشخيص وبعد ستة أشهر من العلاج الكيميائي. أُجريَ التنميط الجيني باستخدام تفاعل البلمرة المتسلسل على تعدد الأشكال HLA-G14-bp. تركز هذه الدراسة على تفاعل ديناميكياتsHLA-Gوالنمط الجيني مع نتائج الاستجابة السريرية، والتي قد تكون استجابة كاملة أوعدم استجابة. **النتائج**:انخفضت مستويات sHLA-Gبشكل ملحوظ بعد العلاج الكيميائي (28.5±15.3نانوغرام/مل عندT0مقابل7.6±6.1 نانوغرام/مل عندT1، $p<0.001$). كانت قيم sHLA-Gالأساسية التي تزيد عن 30 نانوغرام/مل مؤشرًاهامًا على عدم الاستجابة للعلاج، حيث أظهرت حساسية بنسبة 82% وخصوصية بنسبة 70%. ولم تُلاحظ اختلافات جوهرية في توزيع الأنماط الجينية لـ -14 HLA-G bp بين المستجيبين وغير المستجيبين ($p=0.45$). **الأستنتاجات**: يُعد sHLA-Gمؤشرًا حيويًا وديناميكيًا لعبئ المرض؛ وترتبط المستويات الأساسية المرتفعة ارتباطًا وثيقًا بعدم الاستجابة للعلاج الكيميائي، في المقابل لم يُحدد تعدد أشكال HLA-G14-bp كمؤشرمستقل للاستجابة العلاجية في هذه المجموعة.

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Article citation: Khaleel ZK, Al-hasso IKQ, Al-Yozbakee ZM, Al-Badran IM, Khalaf A. Dynamics of Soluble HLA-G and the 14-bp Insertion/Deletion Polymorphism as Predictors of Therapeutic Response in Acute Myeloid Leukemia. *Al-Rafidain J Med Sci*. 2026;10(2):73-78. doi: https://doi.org/10.54133/ajms.v10i2.2861

INTRODUCTION

Human leukocyte antigen G (HLA-G) is a non-classical MHC class I molecule, which is immunomodulatory and is typically expressed in locations that are immunologically privileged, including the maternal-fetal interface to ensure tolerance. The HLA-G gene has seven isoforms formed through alternative splicing; four of them (HLA-G1 - G4) are membrane-bound, and three (HLA-G5 - G7) are soluble isoforms of HLA-G1, -G2, and -G3, respectively. Proteolytic cleavage of the membrane-bound isoforms can also produce soluble HLA-G (sHLA-G) [1,2]. HLA-G is an immune-tolerant

and immune-promoting factor and has proven to be a novel immune checkpoint [3, 4]. It is a strong immunosuppressive mediator and one of the reasons for immune evasion and spreading of tumors in various malignancies. The levels of cancer incidence and mortality are increasing worldwide, with hematologic malignancies accounting for about 10 percent of all newly diagnosed cancer cases worldwide [5, 6]. The structural features of the HLA-G gene are the same as the classical MHC class I molecules, which include eight exons and seven introns. Nevertheless, its genetic polymorphism is limited, mainly due to the terminator codon being in exon 6, which causes most of exon 6 and all of exons 7 and 8 to remain untranslated [7, 8]. In the last 50 years, HLA biology has represented a breakthrough that has made possible life-saving allogeneic hematopoietic transplants even in the case of selected HLA mismatches by expanding the understanding beyond the highly polymorphic classical HLA molecules (A, B, C) to recognize the specialized roles of non-classical HLA molecules (E, F, G) [2, 9-11]. Although a great deal of data is available about the HLA-G expression in solid tumors, there is limited and controversial data on the prognostic role of HLA-G in hematologic cancer and longitudinal studies of sHLA-G dynamics before and after chemotherapy in AML [12,13]. The vast majority of the published research provides only a time point measurement of the same at the time of diagnosis, which does not reflect the dynamics of these immune checkpoints throughout treatment. This study addresses this significant gap by quantifying the levels of preinduction chemotherapy and six months postinduction chemotherapy of sHLA-G and finding out the predictive ability of the HLA-G 14-bp genotype for AML patients' responses to chemotherapy. HLA-G as an immune checkpoint in oncology has received much interest because of its capacity to suppress the cytotoxic action of the NK cells and T lymphocytes [14,15]. Although the findings were first described in the maternal-fetal interface, aberrant expressions of the HLA-G are now defined as important processes involving immune evasion observed in many malignancies. Table 1 demonstrates past related research.

Table 1: Previous related studies about the role of HLA-G in various malignancies

Disease Population	Key Findings HLA-G sHLA-G	14-bp Ins/Del Genotype Findings	Comments Relevance	References
Patients with B-cell chronic lymphocytic leukemia (CLL) (n=126).	Del/del genotype related to the increased surface and soluble HLA G. Plasma of high sHLA G induced apoptosis of NK cells and hampered NK cell lysis.	The del/del genotype was associated with poorer overall survival than ins/del or ins/ins.	Compelling evidence that HLA-G plays a role in immune suppression in CLL; creates a functional impact of high sHLA-G.	[3,7]
47 B-CLL patients	HLA-G percentage positivity of leukemic cells was between 1-54 percent.	No 14-bp genotype data provided	Such results suggest that a worse prognosis in chronic lymphocytic leukemia (CLL) correlates with the expression of membrane-bound HLA-G.	[16]
The patients with the advanced-stage classical Hodgkin lymphoma (cHL) were compared (n= 20) with healthy controls.	The expression of HLA-G protein in the biopsies of the lymph node was also higher in patients as compared to controls.	HLA-G 14-bp polymorphism was also assessed by insertion/deletion polymorphisms though there was little data on its relevance on clinical outcome in this cohort.	Suggests HLA-G participation in immunosuppression in the tumour microenvironment of Hodgkin lymphoma.	[17]
N= 150 Non-Hodgkin Lymphoma (NHL) cases and 100 healthy controls.	The study aimed to determine the relationship between HLA-G 14-bp and HLA-G 14-bp-deletion polymorphism. The frequencies of the genotype were compared, and patient and healthy control groups were taken.	There was a substantially increased risk of NHL (odds ratios >10) and adverse clinical and pathological phenotypes of both the Del/Del and Ins/Del and dominant genotypes and negative consequences of NHL in the form of high lactate dehydrogenase (LDH) and 2-microglobulin concentrations, cytopenias, and NHL relapse.	Indicators suggest that the 14 bp polymorphism can be a genetic risk factor of NHL in some populations, citing ethnic/population variability.	[18]
59 patients undergoing allo-HSCT	Compared pre- and post-transplant soluble HLA G and statistically related to clinical outcome.	HLA-G spermic level or 14 bp genotype of a patient had no significant effect on the risk of acute graft versus host disease (GvHD), relapse, or death.	Refers to the fact that HLA G polymorphism and sHLA G may not necessarily provide any results in the setting of hematopoietic transplantation, which suggests the presence of regulatory complexity	[19]

Although this has been discovered, there is a lack of longitudinal studies of AML that measure HLA-G levels prior to and following standardized chemotherapy. In addition, the level of the sHLA-G at diagnosis has not been clarified as to whether the result is strictly based on the genotype of the germline of the patient or based on the burden of the tumor itself. The proposed research is filled with these gaps since it assesses sHLA-G as a dynamic therapy response indicator and identifies the prognostic value of the 14-bp genotype in adults with AML.

METHODS

Study design and participants

This was a prospective longitudinal study conducted at Al-Sayyab Teaching Hospital between March 15, 2024, and November 20, 2025. The study included 46 adult patients newly diagnosed with AML by a specialist clinician. The diagnosis of AML was established according to the 2016 WHO classification criteria, specifically requiring a diagnostic threshold of >= 20%

myeloblasts in the bone marrow or peripheral blood. To ensure definitive lineage identification and differentiate AML from acute lymphoblastic leukemia (ALL), multiparametric flow cytometry was performed on all samples.

Inclusion criteria

Patients aged 16 years and older; a confirmed diagnosis of AML with >= 20% blasts; and no prior history of chemotherapy or immunotherapy.

Exclusion criteria

Patients were excluded if they were diagnosed with acute promyelocytic leukemia (APL), as identified by the *t*(15;17) translocation. Other exclusion criteria included the presence of active systemic infections at enrollment, a history of other prior malignancies, or pregnancy.

Treatment protocol and response assessment

All participants received standard induction chemotherapy under clinician supervision as prescribed by the attending clinical hematologist in accordance with established institutional guidelines. Clinical response was formally assessed six months following the initiation of induction therapy. A 6-month post-treatment time point was selected to allow stabilization of immune reconstitution following induction and consolidation therapy, thereby enabling meaningful assessment of HLA-G dynamics in relation to disease response and immune escape mechanisms. Patients were categorized into two groups based on their clinical outcome as follows: Complete Remission (CR) is defined as a bone marrow blast count of < 5%, an absolute neutrophil count (ANC) of >= 1x10^9/L, a peripheral platelet count of >= 100x10^9/L, and the total absence of extramedullary leukemia, and Non-Remission (NR) is defined as the failure to meet the criteria for CR.

Sample collection and biomarker quantification

At the time of diagnosis (T0), two separate peripheral venous blood samples were collected from each patient. 3 ml of blood was collected in a plain tube for serum separation to quantify baseline sHLA-G levels. For genotyping, 2 ml of blood was collected in an EDTA tube for the analysis of the HLA-G 14-bp insertion/deletion polymorphism. Following the six-month treatment period, a subsequent 3 ml blood sample was collected in a plain tube from each patient to reassess sHLA-G levels. The study evaluated these biomarkers at two specific time points (T0: at diagnosis (baseline) and T1: 6 months post-induction therapy). The serum level of soluble HLA-G (sHLA-G) was determined by using a validated ELISA kit (sHLA-G ELISA BioVendor Human sHLA-G, Czech Republic). All the samples underwent a duplicate analysis. In this longitudinal model, each patient served as his own control to determine the dynamic variations in the levels of biomarkers as compared to their baseline levels.

Genotyping of HLA-G 14-bp insertion/deletion polymorphism

Standard commercial extraction kit (Miniprep ReliaPrep®, its catalog number: A5081, Promega, USA) used for gDNA extraction. Polymerase chain reaction (PCR) amplification of the HLA-G gene using primers F: 5′-GTGATGGGCTGTTTAAAGTGTCACC-3′ and R: 5′GGAAGGAATGCAGTTCAGCATGA-3′ (Macrogen, Korea) via a thermal device (T100, USA). PCR products were then examined under UV light after being electrophoresed on an agarose gel. DNA size in test tracks was calculated using the DNA ladder (100 bp) in the first track as a reference. The PCR products with sizes of 210 bp were for the deletion allele, and those of 224 bp were for the insertion allele. Products with sizes of 224/210 bp were for the insertion-deletion of HLA-G exon 8. Depending on the size of the fragments, patients were classified under one of three genotype types, which included Del/Del, Del/Ins, or Ins/Ins, as in Figure 1. Thermal cyclic conditions were an initial denaturation phase at 95 °C (5 min), followed by 35 cycles of 94 °C (30 s), 60 °C (30 s), 72 °C (1 min), and a 72 °C (5 min) final extension.

Figure 1: Outcomes of polymerase chain reaction-based 14 base-pair sHLA-G insertion-deletion variant. L: DNA Ladder; Lines: 1, 5, 6,8,9,10,11 and 12 ins/del; Lines:3,4,7 del/del; Line 2: ins/ins.

Data preparation

The most important variables were obtained to perform statistical analysis, such as baseline levels of the soluble form of HLA-G (sHLA-G) before induction chemotherapy, end levels, the level of HLA-G after induction chemotherapy, clinical response status, and the genotype of HLA-G 14-bp insertion/deletion. To enable statistical modeling, the response variable, which is the clinical response, would be coded as a binary response with 1 representing complete remission (CR) and 0 representing non-remission (NR). This design enabled directly comparing the responders to non-responders and

planning comparisons, correlation, and receiver operating characteristic (ROC).

Ethical considerations

The research has been done following the Declaration of Helsinki. The approval of the Institutional Review Board (E/T 67) was achieved. Informed consent was also received in written form before enrolling all the participants.

Statistical analysis

All statistical analyses were performed on SPSS version 28 (IBM Corp., Armonk, NY, USA). Continuous variables were studied as means with standard deviation (SD) in case of a normal distribution and median and the interquartile range (IQR) in case of a non-normal distribution. The Shapiro-Wilk test was used to test the normality of the distribution. Continuous comparisons were made between groups of study using independent t-tests when the sets of study data were normally distributed and the Mann-Whitney U tests when the sets were non-normally distributed. Paired significance based on the comparison of baseline (T0) with follow-up (T1) was performed by the paired t-test. Relations between categorical variables, such as genotype and response to the treatment, were compared by the chi-square test when there were expected counts that were lower than five. The correlation coefficient was used to assess the correlations between the two continuous variables. The receiver operating characteristic (ROC) curves were used to calculate the predictive ability of the concerned parameters, and the area of the curve (AUC) was provided as well. The artificially significant level was defined as a *p*-value below 0.05.

RESULTS

The participants were 46 adult patients with newly diagnosed AML, and the mean age of the participants was 46.2 ± 14.5 years old. After the induction chemotherapy, 27 patients (58.7%) and 19 patients (41.3%) reached complete remission (CR) and non-response (NR). Table 2 is a synthesis of baseline data that includes sex distribution, WBC count, hemoglobin levels, marrow blast percentage, type of AML, and HLA-G 14-bp genotype. Patients with CR also had lower WBC counts (median 52.3 × 10^9/L, IQR 24.0–72.5) and lower marrow blast percentages (57.3 ± 18.4% vs. 70.4 ± 23.8%) than NR patients. However, they were not statistically significant (Mann-Whitney U test of WBC, p= 0.06; independent t test of blasts = 0.08). The sHLA-G level in serum was significantly high at the time of the diagnosis, and it also reduced greatly following induction chemotherapy.

Table 2: Baseline Characteristics of AML Patients (n= 46)

Parameter	All Patients (n=46)	CR (n=27)	NR (n=19)	*p*-value
Age (year)	46.2±14.5	44.1±12.8	49.1±16.4	0.28[a]
Sex (Male/Female)	25/21	15/12	10/9	0.86[b]
WBC (×109/L)	78.4 [38–150]	52.3 [24.0–72.5]	113.6 [65.2–185.4]	0.06[c]
Blast %	62.8±21.5	57.3±18.4	70.4±23.8	0.08[a]
HLA-G Genotype				
Del/Del	18(39)	12(44)	6(32)	
Del/Ins	20(43)	11(41)	9(47)	0.45[b]
Ins/Ins	8(17)	4(15)	4(21)	

Values are presented as frequency, percentage, median [IQR], and mean±SD. [a]Independent t-test; [b] Chi-square test; [c] Mann-Whitney U test.

The level of serum sHLA-G was highly increased at the diagnosis and was reduced significantly after the induction chemotherapy. The average sHLA-G of the entire data sample was 28.5 ± 15.3 ng/mL at baseline and 7.6 ± 6.1 ng/mL after treatment (paired t-test, p= 0.001). CR patients underwent a deeper reduction (stratified on the outcome of the clinical outcome) (21.5 ± 9.8 → 4.5 ± 2.8 ng/mL, paired t-test, p< 0.001), and NR patients had a decline as well (35.4 ± 17.2 → 12.7 ± 6.4 ng/mL, paired t-test, p= 0.002) when stratified as well. A summary of these results is provided in Table 3. The individual patient curves are shown in Figure 2.

Table 3: sHLA-G Levels Before and After Chemotherapy (ng/mL)

Group	Baseline (T0)	Post-treatment (T1)	*p*-value
All Patients (n=46)	28.5±15.3	7.6±6.1	<0.001
CR (n=27)	21.5±9.8	4.5±2.8	<0.001
NR (n=19)	35.4±17.2	12.7 ± 6.4	0.002

Values are presented as mean±SD.

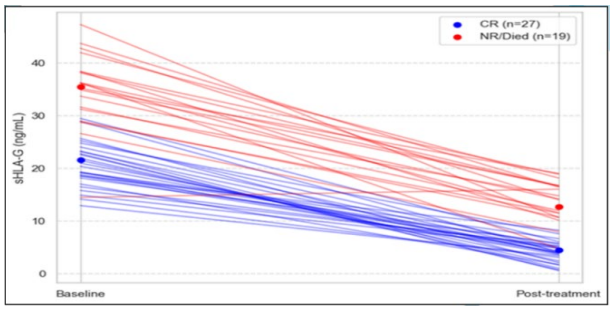

Figure 2: Individual sHLA-G Trajectories (CR vs NR).

The median of the baseline sHLA-G levels also differed significantly between patients who reached CR and NR/died (median 21.5 ng/mL, IQR 14.2–28.9 vs. 35.4 ng/mL, IQR 24.0–48.6; Mann-Whitney U= 134.0, p= 0.001). The analysis of ROC curves indicated that there was fair-to-good predictive power of baseline sHLA-G to treatment response, with the area under the curve (AUC) being 0.78 (95% CI: 0.65-0.91). The sensitivity and specificity with a cutoff value of 30 ng/ml are 82% and 70%, respectively, as indicated in Figure 3. HLA-G genotype treatment response was analyzed with no statistically significant differences. Del/Del patients were the most probable to have a remission rate (66.7 percent),

then Del/Ins (55.0 percent), and Ins/Ins (50.0 percent) (Chi-square test, p = 0.45). The difference in the pretreatment sHLA-G and the post-treatment sHLA-G was also not relevant in terms of genotypes, as indicated in Table 4.

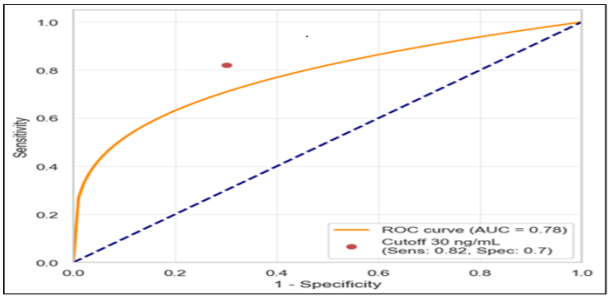

Figure 3: ROC curve for baseline sHLA-G predicting remission (AUC=0.78, 95% CI= 0.65–0.91, Optimal cutoff= 30 ng/mL, Sensitivity= 82%, and Specificity= 70%).

Table 4: Clinical response by HLA-G 14-bp genotype

Genotype	Total	CR (n)	CR Rate (%)	*p*-value
Del/Del	18	12	66.7	0.45
Del/Ins	20	11	55.0	
Ins/Ins	8	4	50.0	

The Spearman correlation analysis indicated that sHLA-G baseline levels were positively related to the marrow blast percentage (0.45, p *p*= 0.002) and WBC count (0.32, p *p*= 0.03) and negatively correlated with hemoglobin (0.28, p = 0.05), as indicated in Table 5.

Table 5: Spearman correlation of baseline sHLA-G with hematologic parameters

Parameter	Spearman ρ	*p*-value
Blast %	0.45	0.002
WBC	0.32	0.03
Hb	–0.28	0.05

On the whole, CR patients were comparatively of a lower baseline sHLA-G, WBC, and blast percentage and marginally higher hemoglobin than NR ones. The summary statistics include the median and the interquartile ranges, which justify the findings of the parametric and non-parametric comparisons (Table 6).

Table 6: Descriptive Statistics of Hematologic and sHLA-G Parameters by Response

Parameter	CR (n=27)	NR (n=19)	*p*-value
Baseline sHLA-G (ng/mL)	21.5 [14.2–28.9]	35.4 [24.0–48.6]	0.001[a]
Post-treatment sHLA-G (ng/mL)	4.5±2.8	12.7± 6.4	<0.001[b]
WBC (×109/L)	52.3 [24–72.5]	113.6 [65–185]	0.06[a]
Blast (%)	57.3±18.4	70.4±23.8	0.08[c]
Hb (g/dL)	10.8±1.9	10.2±2.1	0.21[c]

Values are presented as frequency and mean±SD. [a] Mann-Whitney U test (Median [IQR]); [b] Paired t-test comparing T0 *vs*. T1; [c] Independent t-test.

DISCUSSION

The key result of the present research is that the level of soluble HLA-G (sHLA-G) is remarkably correlated with the clinical outcome of AML patients. We also have seen a significant increase in sHLA-G on diagnosis and a sharp and significant decrease after induction chemotherapy ($p< 0.001$). The association of leukemic blasts in this pathway is also a significant indication that these blasts are a major source of sHLA-G. The positive correlation between baseline sHLA-G and marrow blast percentage proves this hypothesis. It is worth noting that, whereas this correlation is statistically significant (p = 0.002), the correlation coefficient value shows that the relationship between them is of moderate strength (ρ= 0.45). This implies that it is a significant contributor, though it is probably not the only contributor. The tumor microenvironment might have other constituents, such as tumor-associated macrophages or mesenchymal stromal cells [20], that can determine the total serum sHLA-G pool, possibly by being the reason why the correlation with blast percentage is not complete. In addition, mechanistically, when sHLA-G is overexpressed, the sHLA-G prevents the immunosuppressive effect of the microenvironment through binding to ILT2 (CD85j) and ILT4 (CD85d) inhibitory receptors on the natural killer (NK) cells and T-lymphocytes, which effectively inhibits the ability of the immune system to recognize and kill malignant cells. This is in line with our observation that patients who already had the baseline level exceeding 30 ng/mL were much higher in the likelihood of failing in induction therapy; the immune surveillance in these patients must have been impaired before the induction therapy was possible. It was notable that the sHLA-G was a more accurate predictor of therapeutic outcome, compared to the traditional data on the burden of disease in this group of people. Although the white blood cell count and the marrow blast percentage differences between the responders and the non-responders were found to take a direction towards statistical significance (p = 0.06 and 0.08, respectively), they were not found to achieve statistical significance. Conversely, the baseline sHLA-G levels markedly diverged (p = 0.001) between the two groups, which indicates that sHLA-G represents certain immunobiological characteristics of the leukemia, particularly its capacity to avoid immune responses that are not represented by the tumor bulk. Moreover, HLA-G 14-bp deletion/insertion polymorphism did not have any statistical relationship with therapeutic response (p= 0.45). Although the theoretical models indicate that the Del/Del genotype stabilizes mRNA and increases protein expression, whereas the Ins/Ins genotype activates mRNA degradation by the tumor in active AML, anyway, the germline genetic modulation might succumb to the tumor effects. The sharp upregulation of sHLA-G in non-responders is perhaps spurred by the malignancy itself, possibly in either a promoter hypomethylation process or a cytokine-mediated upregulation process as

opposed to the ordinary stability of mRNA afforded by the 14-bp polymorphism. This is consistent with studies conducted by other researchers [6,21], who both observed that even though genetic polymorphisms offer a background risk, they are not necessarily determinative predictors of disease occurrence in complex leukemias. There are a number of weaknesses and shortcomings that must be realized. To begin with, the sample size (n= 46) is relatively small, so statistical power might miss small effects of genotypes. Secondly, no functional assays were done to prove the sHLA-G-mediated inhibition of patient-specific NK or T cells. Lastly, even though the hematologic parameters were measured standardly, patients were not categorized based on the established molecular and cytogenetic risk categories. Thus, it can be concluded that, although sHLA-G has a good predictive value in this cohort, further multivariate studies are justified to establish whether sHLA-G has additional prognostic data beyond already identified molecular risk factors.

Conclusion

Soluble HLA-G is a sensitive and changing biomarker that is used in AML to accurately assess the leukemic load and predict response to therapy. The absolute level of sHLA-G protein in diagnosis, as opposed to the germline genotype of HLA-G 14-bp, was found to be the most important prognostic factor in this cohort. SHLA-G discrete post-treatment normalization should serve as a biochemical sign of remission and thus should be considered useful in the confirmation of minimal residual disease. The next step in work should involve longitudinal assessment of larger, multi-center cohorts that encompass extensive molecular profiling and the exploration of sHLA-G-targeted therapy solutions to circumvent the problem of immune evasion and enhance patient outcomes.

Conflict of interests

The authors declared no conflict of interest.

Funding source

The authors did not receive any source of funds.

Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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