A potential immunological diagnostic marker for acute myeloid leukemia: the expression of the immune checkpoint B and T lymphocyte attenuator

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Introduction Provided the physiologic tumor suppressive role a healthy immune system has, diagnosis of cancer is sometimes also regarded as a diagnosis of an immune dysfunction. Immunoediting refers to the change of immune system response from tumor protection to tumor promotion, favoring the growth of poorly immunogenic clones that have the ability to evade the immune response. Among the mechanisms by which the tumor cells bypass the immune system is immune checkpoints. Which are immune inhibitory pathways responsible for self-tolerance and limiting self-tissue damage by controlling the magnitude and duration of the immune reaction.

Objective Studying the expression of the novel immune checkpoint BTLA in patients with AML and evaluate its clinicopathological and diagnostic significance.

Patients and methods We investigated the expression of BTLA gene in 60 AML cases and 15 healthy controls using RT-PCR.

Results The current study revealed that significant up regulation of BTLA mRNA expression in AML patients as in comparison with the control group (P=0.024).

Discussion These results provide a basis for the perception that BTLA upregulation is involved in inhibition of antitumor immunity and that high BTLA expression level can be made use of as a diagnostic marker in patients with AML.

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Introduction

Immune response to cancer cells occurs in one of two ways, either as a reaction against tumor-specific or against tumor-associated antigens both are molecules expressed in a different way in cancer than normal cells [1].

Cancer immune response involves two major stages, namely, the priming and effector phases. During priming, the antigen-presenting cells (APCs) activate the T cells. The tumor antigens, which are tumor-specific antigens or tumor-associated antigens, which include molecules expressed in a different way in cancer than normal cells [1], are obtained from dying cancer cells. Danger signals including type I interferons, are produced and act as adjuvants to increase the immune response [2]. During the effector phase, antigens together with danger signals cause the maturation of DCs. DCs produce three signals to induce effector T cells. 1. Via antigen presentation on MHC molecules on DCs to T cell receptors 2. Co-stimulatory or co-inhibitory molecules. 3. Production of a number of cytokines. The main effector cells for the destruction of tumor cells are cytotoxic T lymphocytes [3].

Tumors escape the immune system by various mechanisms resulting in immunoediting. The mechanisms interfering with antitumor immune responses fall into the following categories: (a) the first is the defective tumor antigen presentation, as tumor antigens may be present in different forms because of genetic instability or tumor mutation. Epitope-negative tumor cells may thus escape the immune response [4]. (b) The second is tumor cell resistance. As the cancer pathologic process progresses, the tumor cells become more genomically unstable, which increases their immune resistance [5]. (c) The third is absence of activating mechanisms owing to the absence of costimulatory molecules such as CD28 and CD80. Thus, tumor cell-induced apoptosis is minimal [6]. (d) The fourth is activation of immune checkpoints. The activation of immune checkpoints expressed on activated T cells results in inhibition of T-cell activation when bound to specific ligands on tumor

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cells/APC, particularly against the cytotoxicity caused by antigen-specific T cells, thus suppressing antitumor immunity [7]. Among the immune checkpoints, cytotoxic T-lymphocyte-associated antigen 4 and programmed cell death protein 1 are commonly studied as a result of promising clinical applications of monoclonal antibodies against them [8]. The binding of these inhibitory receptors results in a decrease in the protective role of antigen-specific CD8+ T cells against tumor cells. Accordingly, in cancer a link has been established between the expression of co-inhibitory receptors and the T-cells dysfunction, thus the blockage of these receptors has revealed clinical promise [9].

BTLA is another co-inhibitory molecule, having a negative regulatory effect on T-cell activation and is expressed on a variety of immune cells including B-cells, T-cells, and dendritic cells [10]. BTLA interacts with herpesvirus entry mediator, a member of the necrosis factor receptor family. Its ligation with herpesvirus entry mediator causes suppression of T-cell development and dampened immune response, as well as reduced cytokine production, including inhibition of INF-γ, IL-2, IL-4, and IL-10 [11]. Limited data are published concerning the biological role of BTLA expression in acute myeloid leukemia (AML). Hence, the purpose of this study was to investigate the clinical significance of BTLA expression as well as its diagnostic value in AML patients since checkpoint inhibition is a promising therapeutic goal.

AML is a hematopoietic stem cell disorder pigeonholed by the expansion of undifferentiated myeloid progenitors. It is heterogeneous from the clinical, cytogenetic, and molecular points of view. Nearly 30% of cases carry frequent chromosomal abnormalities [12]. Management of patients with AML takes into consideration the heterogeneity of the disease, as well as incorporation of several predictive factors such age, performance status, and recognition of cytogenetic risk factors [13]. It is aggressive and devastating, showing primary response to chemotherapy but when not eradicated in the first attempt becomes progressively more resistant to treatment [14]. The average 5-year survival rate for patients with AML is ~27% [13].

Patients and methods

Patients

A total of 60 newly diagnosed patients with AML were recruited from the Internal Medicine Department, Clinical Hematology and Stem Cell Transplantation Unit, Ain Shams University Hospitals, Cairo, Egypt. The diagnosis was based on the morphologic findings from Wright–Giemsa-stained smears prepared from bone marrow aspirates and immunophenotyping analyses of leukemic cells. Moreover, 15 healthy, age-matched and sex-matched volunteers were also involved in the study as the healthy control group. The study was approved by the Ethical Committee of Research, Faculty of Medicine, Ain Shams University, and was conducted consistent with the Declaration of Helsinki. Informed consent were also signed.

Blood sampling

Peripheral blood samples were collected prior to receiving any treatment and collected on vacutainer tube containing Na_2 EDTA for determination of complete blood count and for total RNA purification.

Methods

Complete blood count determination

Complete blood count determination was carried out by Z2TM Coulter Counter (Coulter Electronics, Hialeah, Florida, USA).

RNA isolation and real-time quantitative PCR

The QIAamp RNA blood mini kit (Qiagen, Hilden, Germany) was used for total RNA extraction from human whole blood according to the manufacturer's protocol. Complementary DNA was synthesized using High-Capacity Complementary DNA Reverse Transcription kit (Applied Biosystems, Foster City, California, USA) and stored at −20°C until use. Gene expression analysis was performed with the Rotor-Gene Q Real-Time PCR cycler (Qiagen) using standard thermal cycling conditions and Taqman assays specific for BTLA. GAPDH was the endogenous control used for normalization of data. The expression levels in unknown samples were normalized and analyzed by the $2^{-\Delta\Delta Ct}$ method where $\Delta\Delta Ct=(Ct_{target\ gene}-Ct_{GAPDH})$ sample−$(Ct_{target\ gene}-Ct_{GAPDH})$ calibrator.

Statistical analysis

Data were analyzed using IBM SPSS Statistics, version 23 (IBM Corp., Armonk, New York, YSA) and MedCalc, version 18.2.1 (MedCalc Software, Ostend, Belgium). Continuous numerical variables were presented as mean and SD, and intergroup differences were compared using the unpaired *t* test. Nonparametric data were shown in the form of as median and interquartile range, and between-group differences were compared using the Mann–Whitney

Table 1 Clinical data of the studied groups

Characteristics	Control group	AML group
Sex	7 males/8 females	24 males/36 females
Age (years)Φ	46.9±6.3	53.4±12.9
Hemoglobin (g%)	14.5 (13.9–15.6)	7.9 (5.9–9.6)[a]
WBCs (×10^{3}cells/μl)	5.1 (4.1–7.8)	17.6 (3.2–43.2)
PB blasts (%)	–	34 (10–62)
BM blasts (%)	–	36 (25–71)
BTLA fold expression	1.14 (0.70–1.15)	1.77 (0.96–3.68)[b]

Data are median (25th and 75th centiles quartiles), Φ=mean±SD. AML, acute myeloid leukemia; BM, bone marrow; BTLA, B and T lymphocyte attenuator; PB, peripheral blood; WBC, white blood cell. [a]Significantly different from healthy control group at *P* value less than 0.001. [b]Significantly different from healthy control group at *P* value of 0.024.

U test. Correlation was estimated using the Spearman rank correlation. Two-sided *P* values less than 0.05 were considered statistically significant. Receiver-operating characteristic curve analysis was used to examine the diagnostic value of gene expression [15].

Results

Clinical data of the studied groups

Clinical data of the studied groups are presented in Table 1.

B and T lymphocyte attenuator expression level and its diagnostic value in patients with acute myeloid leukemia

Patients with AML showed significantly upregulated BTLA expression in comparison with the healthy control group (Table 1). Patients' age or gender did not have an effect on Its expression level. We further evaluated its diagnostic value using receiver-operating characteristic curves, where it showed moderate diagnostic value with area under the curve, diagnostic sensitivity, and specificity of 0.70 (95% confidence interval: 0.573–0.792, *P*=0.002), 51.7, and 100%, respectively, for a cutoff criterion of more than 1.537 (Fig. 1).

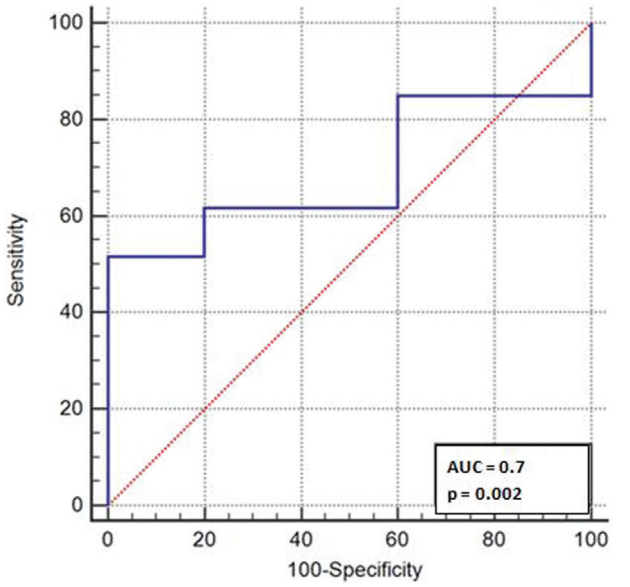
Correlation coefficients between B and T lymphocyte attenuator expression levels and complete blood count parameters in patients with acute myeloid leukemia

A strong negative correlation was observed between BTLA expression and both hemoglobin (r=–0.620, P<0.001; Fig. 2) and bone marrow blasts (r=–0.331, P=0.01; Fig. 3) in patients with AML.

Discussion

A healthy immune system should have the ability to suppress tumor cells; however, tumor cells possess a number of mechanisms to escape immune surveillance, and among those mechanisms, one is immune

Figure 1



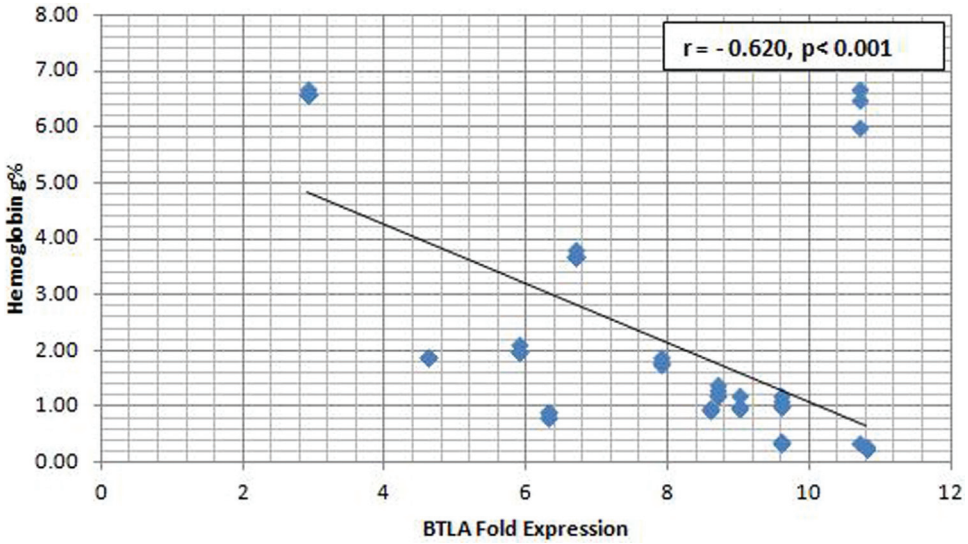
Receiver-operating characteristic (ROC) curve for evaluating the diagnostic value of BTLA expression. BTLA, B and T lymphocyte attenuator.

checkpoints. There is a recent interest in the study of immune checkpoints because of their potential use in the diagnosis, prognosis, and as a potential treatment target for different cancers. Although considerable research is now available about immune checkpoints in solid tumors, research is still limited about their role in hematologic malignancies [1,8].

Side by side with cytotoxic T-lymphocyte-associated antigen 4 and programmed cell death protein 1, novel immune checkpoint molecules on T cells are being discovered constantly, and BTLA is one of them. BTLA (CD272) is another inhibitory receptor member of CD28 superfamily. On the human chromosome, it is located in chromosome 3 in q13.2 and encodes a type I glycosylated transmembrane protein composed of 289-amino acid. BTLA was expressed on mature lymphocytes including B cells, T cells, DCs, as well as macrophages [16].

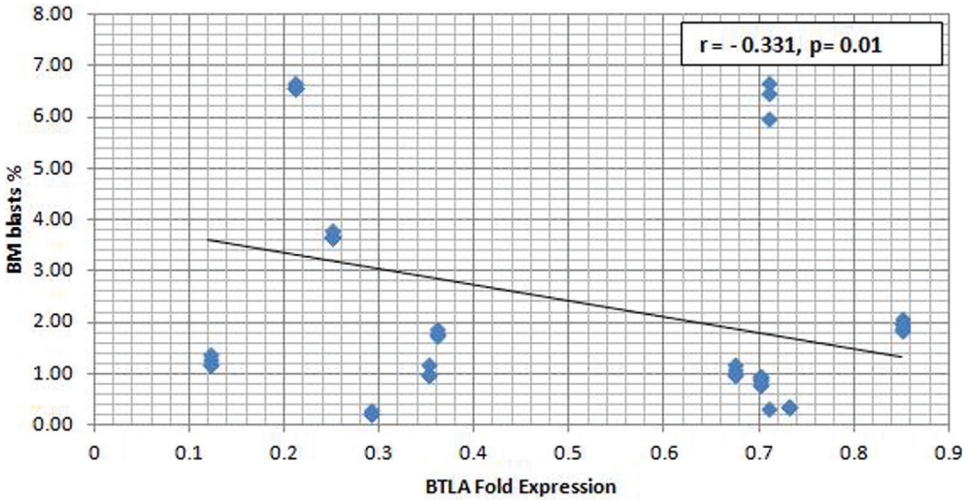
The findings in this study have shown that BTLA gene expression was significantly up-regulated in the studied sample of patients with AML in comparison with the studied healthy control group. It has also shown that this checkpoint is of moderate value in AML diagnosis. The upregulation of BTLA genes results in BTLA signaling into T cells through the phosphatase SHP-1, decreased T cell receptors signaling, as well as the mobilization of preformed CD40. Thus, BTLA overexpression deprives B cells from the T helper signals and it might be suggested that BTLA serves as a cell-extrinsic suppressor of B cell development

Figure 2



Correlation of BTLA fold expression with hemoglobin (g%) in patients with AML (*n*=60). Each individual value is represented by a symbol (&z. squf;). *r*=Spearman's rho. AML, acute myeloid leukemia; BTLA, B and T lymphocyte attenuator.

Figure 3



Correlation of BTLA fold expression with BM blasts (%) in patients with AML (*n*=60). Each individual value is represented by a symbol (&z.squf;). *r*=Spearman's rho. AML, acute myeloid leukemia; BM, bone marrow; BTLA, B and T lymphocyte attenuator.

[14,17,18]. Consequently, BTLA is regarded an important player in the immune editing process that helps in development and progression of cancer.

The 5-year survival rate of patients with AML treated with the current treatment modalities is only 27% [18]; thus, the search for new trends toward better survival is mandatory. In April 2019, Junshi Biosciences declared that the premier anti-BTLA mAb, TAB004/JS004, has received an approval for clinical trial by FDA [19–21].

Although the idea of exploiting the immune system to treat cancer dates back to the early days of immunology research, this idea was hampered by the false belief that tumor cells were only weakly immunogenic. Recently, this idea was modified when it was proven that the immune system does recognize cancer cells, yet tumor cells manage to evade immune surveillance. In the case of hematological malignancies, the deeper understanding of tumor immunology will enable a number of promising immunotherapies. The immunology of hematological tumors, not only AML, trails the discoveries in solid tumors. Nevertheless, research about immunotherapy treatments potentially applicable to AML is growing [22]. Accordingly, it is considered of paramount importance to provide a more profound explanation of the immunology of AML to design, select, and optimize diagnostic

tools and immunotherapies for this resistant malignancy.

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Conflicts of interest

There are no conflicts of interest.

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