Circulating Biomarkers for B-Cell Progenitor Acute Lymphoblastic Leukemia

Subjects: Hematology

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Acute lymphoblastic leukemia (ALL) is a hematological disease characterized by the dysfunction of the hematopoietic system that leads to arrest at a specific stage of stem cells development, suppressing the average production of cellular hematologic components. BCP (B-cell progenitor)-ALL is a neoplasm of the B-cell lineage progenitor. BCP-ALL is caused and perpetuated by several mechanisms that provide the disease with its tumor potential and genetic and cytological characteristics. These pathological features are used for diagnosis and the prognostication of BCP-ALL. The BCP-ALL diagnostic protocol is well established. Firstly, it is necessary to demonstrate ≥ 20% lymphoblasts in bone marrow (BM) based on a BPM. Second, a hematopathological review is performed; it comprises a morphological assessment, and flow cytometric and genetic characterization.

biomarkers circulating acute lymphoblastic leukemia BCP-ALL

1. Introduction

Leukemias are a group of hematological diseases characterized by an abnormal cell population suppressing the average production of cellular components of the hematopoietic system [1]. There are four main subtypes of leukemia that have been identified based on their evolution time and hematological lineage: acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), acute myelogenous leukemia (AML), and chronic myelogenous leukemia (CML) [2]. ALL and CLL are the most common leukemia types in children and adults, respectively [3][4].

ALL is a malignant disorder that occurs when typical lymphoid cell development malfunctions due to arrest at a specific stage of development; the name and classification and based on the dysfunctional stage [5]. ALL can develop from primitive precursor cells with multilineage potential from the two types of lymphoblasts: B or T. These cells proliferate uncontrollably in bone marrow (BM) and peripheral blood (PB) [1][6][7][8] and interfere with the functions of normal blood cells [8][9]. Of the two kinds of lymphoblasts, it is more common for ALL to originate from B-cell progenitor (BCP-ALL), representing about 80% of cases; the remaining 20% originate from T-cell progenitors (TCP-ALL) [10].

According to the World Health Organization (WHO), BCP-ALL is defined as a neoplasm of precursor lymphoid cells committed to the B cell lineage, typically composed of small-to-medium-sized blast cells with scant cytoplasm, moderately condensed to dispersed chromatin, and inconspicuous nucleoli [5]. BCP-ALL diagnosis involves clinical

and laboratory tests. BCP-ALL symptoms indicate blast infiltration in the bone marrow, lymphoid system, and extramedullary sites and may include fatigue or lethargy, constitutional symptoms, dyspnea, dizziness, infections, and easy bruising or bleeding [11]. A BM aspirate is mandatory for laboratory tests to confirm the diagnosis, demonstrating ≥ 20% bone marrow lymphoblasts [12]. In addition, clinicians can examine cell morphology, immunophenotyping, and genetics and integrate these measures according to the classification established by the WHO [5]. BCP-ALL has multiple subtypes characterized by immunophenotype B-I (pro-B), B-II (Common), B-III (pre-B), and B-IV (B—mature Burkitt type) [5]. Recently, the nomenclature of leukemia with genetic abnormalities has shifted from focusing on cytogenetic alterations to molecular events, and new genetic entities have been added. The WHO classification in 2022 is based on clinical–biological entities defined by cytogenetic alterations that confer different prognoses. The United Kingdom ALL copy-number alteration (UKALL-CNA) classification has been incorporated, which distinguishes three genetic risk groups [13].

The peak incidence of ALL is from 2 to 5 years of age. It is rare in adults, but it can be found after 50 years of age. BCP-ALL constitutes approximately 80–85% of total cases of ALL [14][15][16]. Many factors affect the prognosis of BCP-ALL, such as age, the white blood cell (WBC) count, clinical features at diagnosis, cytogenetic abnormalities, pharmacodynamic and pharmacogenetic characteristics of normal patient cells, early response to therapy, and the results of the measurements of minimal/measurable residual disease (MRD: the presence of leukemic cells at specific time points in bone marrow or even in peripheral blood circulation) [17][18][19][20][21][22]. The prognostic implication of cytogenic abnormalities relates to poorly regulated signaling pathways [5][23], which can affect leukemogenesis and directly influences ALL development [24][25].

The above-mentioned factors help determine a “risk stratification” classification for BCP-ALL. This classification determines the treatment regimen and intensity that will be settled to treat BCP-ALL. This classification has contributed to a marked improvement in the prognosis of patients with leukemia [19][26][27].

As mentioned before, a BM puncture (BMP)/aspirate is needed to diagnose BCP-ALL. However, because of its invasive nature, potential side effects, and expensive procedures, there is a real need for alternative measures and molecular markers for the effective diagnosis and prognosis of pediatric patients with BCP-ALL [14][28]. Suitable substitutes for BMP are molecular biomarkers found in peripheral blood; collecting blood produces minimal trauma, and biomarkers can be measured relatively easy and early in the disease [29][30][31]. Nevertheless, the mentioned replacement must fulfill the biomarker standards.

In oncology, a biomarker is any measurable indicator that demonstrates the presence of malignancy, tumor behavior, prognosis, or responses to treatments [32]. Among the myriad of biological materials circulating in the bloodstream, the most promising biomarkers include circulating tumor cells, cell-free DNA (cf-DNA) and RNA, proteins and metabolites, and extracellular vesicles (EVs) [33].

2. Diagnostic Biomarkers for BCP-ALL

The BCP-ALL diagnostic protocol is well established. Firstly, it is necessary to demonstrate ≥ 20% lymphoblasts in BM based on a BPM. Second, a hematopathological review is performed; it comprises a morphological assessment, and flow cytometric and genetic characterization. Once these studies are completed, a diagnosis according to the WHO classification can be made [12]. The BCP-ALL classification integrates morphology, immunophenotyping, and genetics/cytogenetics. There are no morphological features to distinguish between the BCP-ALL and TCP-ALL. Nevertheless, some lymphoblast characteristics are relevant: scant cytoplasm, size, and shape, wide relatively dispersed chromatin, and nuclear, and nucleolar peculiarities [34]. The immunophenotyping in BCP-ALL show some markers as almost always positive, namely CD19, cCD79a, cCD22, CD22, CD24, PAX5, and TdT; CD20, CD34, CD13, and CD33 expression is variable. Finally, among the genetic abnormalities BCR—ABL1, KMT2A-rearranged, ETV6-RUNX1, hyperdiploidy, hypodiploidy, IGH/IL3, TCF3-PBX1, BCR-ABL1-like, and iAMP21 can be mentioned [5].

The following circulating biomarkers have shown promise for either the diagnosis or prognosis of the illness.

2.1. Proteins Type BCP-ALL Biomarkers

Proteins are helpful biomarkers because they have multiple crucial functions, making them central in biological systems [35][36]. In an individual manner, tumor necrosis factor α (TNF-α) is among the most studied biomarkers for ALL. TNF-α is a cytokine that induces apoptosis by inhibiting the activity of caspases [37]. Ahmed et al. evaluated its levels in serum from adult patients with ALL, comparing patients who received chemotherapy to those who did not (**Table 1**). Based on receiver operating characteristic (ROC) curve analysis, TNF-α showed good ALL diagnostic utility, an area under the ROC curve (AUC) of 0.94, a sensitivity of 91.7%, and a specificity of 100% [3]. These reports are consistent with the findings reported by Aref et al., who found an increase in TNF-α levels among patients newly diagnosed and in remission compared with controls [38]. Ahmed et al., was able to confirm this using an ROC curve, even though Aref's cohort of patients had both BCP-ALL and BCP-CLL. These findings indicate that TNF-α can be a valuable tool in diagnosing ALL [3][38][39]. **Table 1** summarizes the markers whose diagnostic values for ALL and/or BCP-ALL have been determined.

Table 1. ROC values of diagnostic biomarkers for BCP-ALL.

Biomarker	Blood Sample	Leukemia Type	Area under the ROC Curve	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Reference
Smad 7	Serum	ALL	0.81	63	100	100	73	[16]
TGF-β1	Serum	ALL	0.79	57	93	89.5	68	
Smad 7 TGF-β1 miR-181a	Serum	ALL	-	100	93	93.7	100	
IGF-I	Serum	ALL	-	60.6	73.3	-	-	[40]

Biomarker	Blood Sample	Leukemia Type	Area under the ROC Curve	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Reference
IGF-II	Serum	ALL	-	72.2	73.3	-	-	
IGFBP-2	Serum	ALL	-	72.2	86.7	-	-	
IGFBP-3	Serum	ALL	-	93.9	93.9	-	-	
Anti-9-0AcSGs	Serum	ALL-GI	-	98.9	92.1	96.8	97.2	[41]
Anti-9-0AcSGs	Serum	ALL-GI	-	96.8	95.9	96.8	95.9	
PF4 CTAP-II	Serum	ALL	-	91.8	90	-	-	[7]
C3f	Serum	AL	0.99	97	100	-	-	[42]
TNF-α	Serum	ALL	0.94	91.7	100	-	-	[3]
Survivin	Serum	ALL	0.98	90	80	-	-	
p53	Serum	AL	0.8	52	100	-	-	[43]
EGFR	Serum	AL	0.93	73.9	95.8	-	-	
Pseudouridine	Serum	ALL	-	90	97.5	-	-	[44]
ADAM 17	Plasma	BCP-ALL	0.98	100	100	-	-	[45]
ATG3	Plasma	BCP-ALL	0.95	100	100	-	-	
AC133 *	Whole blood	ALL	-	100	100	100	100	[46]
miR-181a	Serum	ALL	0.93	86.5	93.3	92.8	87.5	[16]
miR-146a	Plasma	BCP-ALL	1	100	100	-	-	[9]
mRNA Survivin	Whole blood	BCP-ALL	0.85	95	95	-	-	[47]
mRNA HLA-G	PBMC	ALL	-	74	100	-	-	[48]
miR-125b-1	Serum	ALL	0.85	83.7	100	-	-	[49]
miR-203	Serum	ALL	0.87	97.7	87	-	-	
miR-100	PBMC	ALL	0.87	82.7	100	-	-	[15]

Biomarker	Blood Sample	Leukemia Type	Area under the ROC Curve	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Reference
miR-196a	PBMC	ALL	0.537	46.6	100	-	-	[50]
miR-146a	PBMC	ALL	1	100	100	-	-	
miR-511	Plasma	BCP-ALL	1	100	100	1	1	
miR-34a	Plasma	BCP-ALL	0.98	92	100	1	0.70	
miR-22	Plasma	BCP-ALL	0.91	79	100	1	0.54	
miR-26a	Plasma	BCP-ALL	0.91	79	100	1	0.47	
miR-221	Plasma	BCP-ALL	0.92	83	100	1	0.54	
miR-223	Plasma	BCP-ALL	0.93	89	100	1	0.64	
miR-21	PBMC	ALL	0.565	44	55	-	-	[51]
miR-26	PBMC	ALL	0.464	54	50	-	-	
miR-148a	PBMC	ALL	0.719	74	79	-	-	
miR-133b	PBMC	ALL	0.669	70	60	-	-	
miR-24	PBMC	ALL	0.785	72	81	-	-	
miR-92a	PBMC and plasma	ALL	0.99	-	-	-	-	[52]
miR-92a	Plasma	ALL	0.755	41.5	100	100	36.7	[6]
miR-638	Plasma	ALL	0.86	54.7	100	100	42.9	
miR-125b	PBMC	ALL	0.99	98	96.7	-	-	[53]
mRNA-Bcl-2	PBMC	ALL	0.9	96.7	70	-	-	
miR-128b	PBMC	ALL [16]	-	75	87.5	-	-	[54]
cf-DNA levels	Plasma	ALL, AML	0.79	65	100	-	-	
cf-DNA integrity	Plasma	ALL, AML	88	78	90	[16]	-	[55]

R-181a in
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proliferation, differentiation, apoptosis, adhesion, and migration. To activate Smad proteins, a family of receptors is required to undergo phosphorylation and activation. These receptors belong to the kinase domain type I TGF-β, which play a crucial role in controlling the TGF-β signaling pathway. The results showed resistance to the growth inhibitory and apoptotic effects of the TGF-β1 signaling pathway due to elevated Smad 7 levels, thus meaning that the TGF-β1 signaling pathway showed to be involved in ALL pathogenesis [16].

ROC: Receiver operating characteristic; PPV: positive predictive value; NPV: negative predictive value. ALL: acute lymphoblastic leukemia; BCP-ALL: B-cell precursors ALL; AML: acute myeloid leukemia; GI: Group I; GII: Group II; PBMC: peripheral blood mononuclear cell. * Value of AC133 expression to predict cases of poor prognosis.

The presence of autoantibodies VDAC1 (voltage-dependent anion-selective channel 1) ($p < 0.05$) and α-enolase ($p < 0.05$) has also been reported as overexpressed in the serum of patients with BCP-ALL [14]. Researchers have postulated that the presence of these proteins could be immunogenic at the early stages of the disease (although their mechanism remains unclear). Because of their functions, α-enolase and VDAC1 may be used as auxiliary tools for the diagnosis and immunological surveillance, treatment, and prognosis of pediatric BCP-ALL [14].

A valuable method for evaluating multiple proteins is “proteomics”, which evaluates the proteome. The proteome is a group of proteins expressed in a cell, characterized by their distinct localization, post-translational modifications, interactions, and turnover [35]. In leukemias, aberrant protein expression patterns can identify the pathogenesis of the disease, post-translational modifications, origin, and the differentiation of leukemic cells [7][35][36][56]. If successful, the study of one or a small number of proteins contributes to the understanding of the pathogenesis of disease; understanding the dynamics of the human proteome is crucial for developing biomarkers to be used as measurable indicators for disease severity and progression, patient stratification, and drug development. To distinguish between hematological malignancies with a proteome profile, researchers evaluated plasma from 107 patients older than 18 years with AML, ALL, or APLL, and 144 patients with lymphomas by using a proximity extension assay (PEA) [56]. PEA is a technology that translates protein information into actionable knowledge by linking protein-specific antibodies to DNA-encoded tags [57]. Regarding AL, the authors reported von Willebrand factor (vWF), SYND1, TNF-RSF6B, MPO, VIM, TNF-R1, IL-6, CTSD, and FURIN levels were remarkably higher compared to controls (fold change [FC] > 2; p-values < 1.0×10^{-40}); meanwhile, ADAM-TS15 (FC = 0.43; P = 3.34×10^{-40}) levels were lower. Moreover, the most differentiating proteins between ALL, AML, APL, and controls were TCL1A, CD27, and CD48. They did not find significant protein expression differences between of AML and ALL, perhaps due to the small number of patients. Therefore, those proteins can distinguish between leukemia, healthy patients, and other hematological malignancies. The functions of altered proteins were associated with hemostasis, inflammation, and cell-to-matrix integration [56][58][59][60]. Speaking of hemostasis, vWF helps bind platelets to vascular injury sites after a proteolytic reaction regulated by FURIN [56][60]. These data are consistent with increased vWF and FURIN levels because thrombocytopenia and blood coagulation disturbances are crucial in acute leukemia. Similarly, the expression of the members of the TNF-R superfamily is involved in the inflammatory response and contributes to cell differentiation and survival [56][61].

In a study using high throughput technologies for proteins, Zhu et al. evaluated exosomal proteins in plasma from the blood samples of patients with BCP-ALL [45]. After isolating exosomes, they performed proteomic analysis by mass spectrometry and identified 342 differentially expressed proteins associated with Notch and the autophagy pathways, confirmed by Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis. Because of this analysis and their potential role in BCP-ALL, the authors selected ATG3 and ADAM17 as candidate biomarkers for BCP-ALL progression. In the study, both proteins were overexpressed in patients with BCP-ALL, as verified by Western blotting. A high AUC value calculated for ADAM17 (AUC = 0.989, 95% confidence interval (CI): 0.960–1.018, $p < 0.0001$) and for ATG3 (AUC = 0.956, 95% CI: 0.885–1.026, $p < 0.0001$), indicating their potential as diagnostic biomarkers for BCP-ALL [45].

In another study, the authors evaluated the peripheral lymphocytes of 15 children diagnosed with ALL by using 2D electrophoresis, mass spectrometry, and identification in silico [4]. Among the 15 differentially expressed proteins, they could only identify eight. Glutathione S-transferase P (GSTP) ($p < 0.01$) and prohibitin (PHB) ($p < 0.01$) were upregulated. Peroxiredoxin 4 (PRDX4) ($p < 0.01$), the 60S acidic ribosomal protein P0 ($p < 0.01$), pyridoxine-5′ -phosphate oxidase ($p < 0.01$), triosephosphate isomerase 1 ($p < 0.05$), cytoplasmic actin ($p < 0.01$), and hypothetical protein FLJ26567 ($p < 0.01$), were downregulated; it is theorized that the expression levels of this last protein depends on the cell type. Although the authors were not able to speculate on the specific role of each protein in leukemogenesis, the whole panel of proteins demonstrated diagnostic value [4]. Similarly, in children with ALL, Shi et al. evaluated protein differentiation in serum blood samples. Mass spectrometry, bioinformatic analysis, and additional validation with immunoassays revealed that platelet factor 4 (PF4) ($p = 1.54 \times 10^{-7}$) and connective tissue activating peptide III (CTAP-III) ($p = 7.19 \times 10^{-8}$) were downregulated, and the fragments C3a-8137 ($p = 5.35 \times 10^{-5}$) and C3a-8937 ($p = 5.13 \times 10^{-5}$) were upregulated. Together, these proteins showed a sensitivity of 96% and a specificity of 98% for ALL diagnosis, implicating them as potential biomarkers for pediatric-ALL [7].

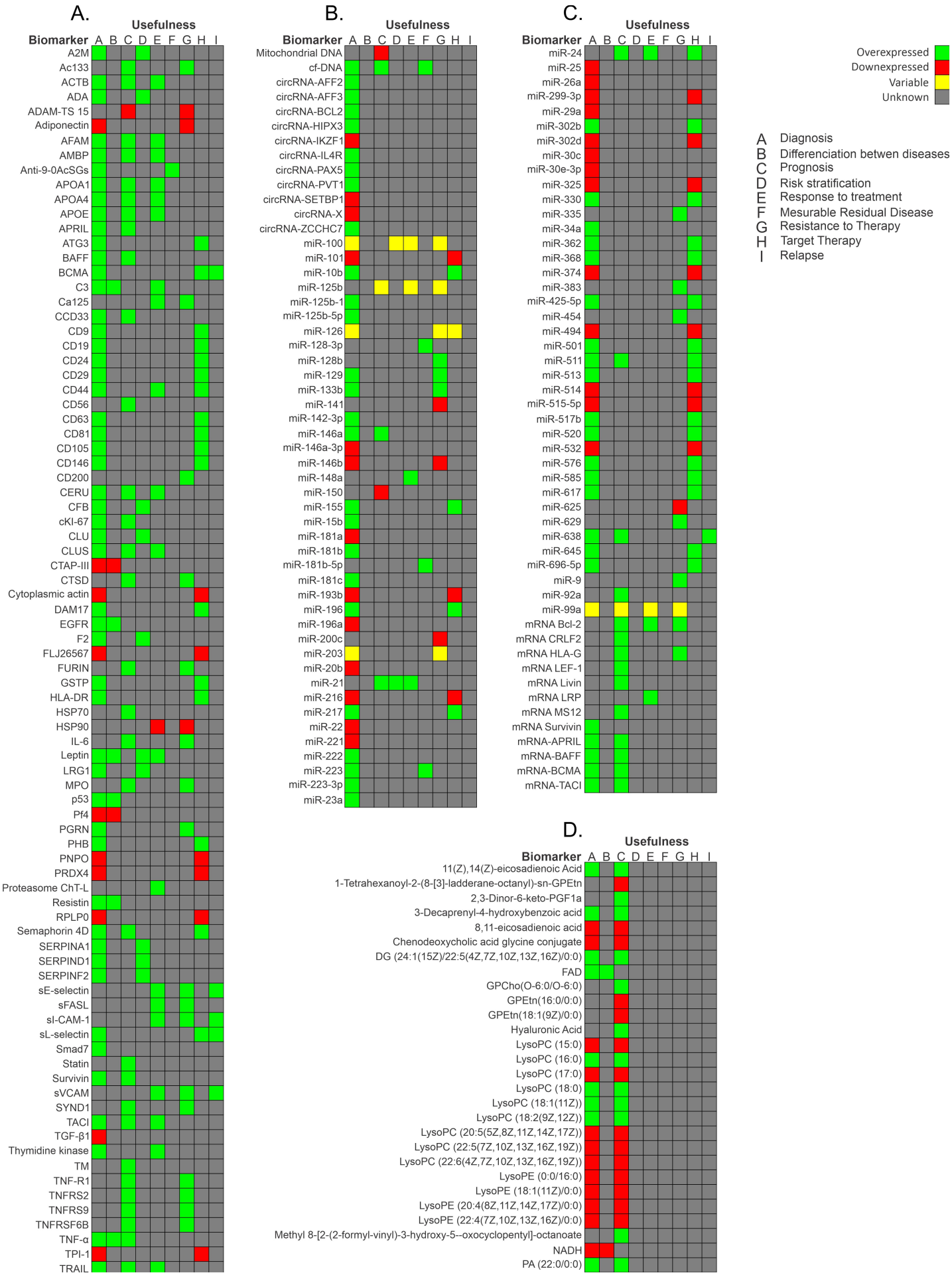
2.2. RNA Type BCP-ALL Biomarkers

RNA can generate numerous proteins through protein-coding regions. Most genomic sequences can be transcribed into protein-coding RNA. In contrast, the non-coding parts are transcribed to produce non-coding RNAs (ncRNAs) [62][63]. Based on their size, location, and interacting partners, ncRNAs are classified into several types: transfer RNAs (tRNAs), ribosome RNAs (rRNAs), small nucleolar RNAs (snoRNAs), small nuclear RNAs (snRNAs), microRNAs (miRNAs), small interfering RNAs (siRNAs), long non-coding RNAs (lncRNAs), circular RNA (circRNAs), Piwi-interacting RNAs (piRNAs), and enhancer RNAs (eRNAs) [62].

It has been discovered that numerous microRNAs are essential in the initiation, progression, and metastasis of cancer [64]. miRNAs are stable in serum and display distinct expression patterns in healthy individuals and those with cancer. Hence, they are suitable biomarkers for cancer detection and prognosis [32]. Furthermore, one miRNA can modulate the expression of several genes, and there are many miRNA expression patterns in patients with ALL [65]. In a study carried out in Mexico, the authors evaluated the diagnostic usefulness of the circulating miRNA expression profile in plasma samples from patients with BCP-ALL. miR-511 showed the highest mean overexpression (FC: 159.5, $p = 0.002$), while miR-199a-3p was the most under-expressed (FC: −13.48, $p < 0.001$). ROC curves analysis provided good values for miR-511 (cut-off = 9.458, specificity 1, sensitivity 1, AUC = 1), miR-34a (cut-off = 7.179, specificity 1, sensitivity 0.92, AUC = 0.98), miR-222 (cut-off = −0.1325, AUC = 0.91), miR-26a (cut-off = 2.073, AUC = 0.91), miR-221 (cut-off = −0.1861, AUC = 0.92), and miR-223 (cut-off −4.309, specificity 1, sensitivity 0.89, AUC = 0.93). Moreover, pathway analysis revealed that mainly Wnt, MAPK, TGF-beta, p53, Jak-STAT, NOTCH, and B- and T-cell receptor signaling pathways are activated by the evaluated miRNAs (miR-511, miR-19b, miR-195, miR-565, miR-34a, miR-222, miR-363, miR-181a, miR-181c, miR-199a-3p, miR-340, miR-335, miR-99b, miR-221, miR-744, miR-223, miR-26a, miR-224, and miR-151-3p). These miRNAs have value as markers for diagnosis and to identify therapeutic targets for BCP-ALL [50].

CircRNAs are a type of non-coding RNA and represent a recent research hotspot in the field of RNA. circRNAs form covalently closed loop structures with neither 5′–3′ polarities nor polyadenylated tails and lengths between 100 to thousands of nucleotides [66][67]. circRNAs can modulate miRNA-target expression, acting like miRNA axes. Moreover, they can interact with RNA-binding proteins and regulate cellular processes [68].

Circular RNA (circRNA) expression in PBMCs of pediatric patients with BCP-ALL has also been evaluated [68]. After the comparison of circRNA expression profiles in B- and T-cells, and monocyte populations, which was validated by reverse transcriptase quantitative polymerase chain reaction (RT-qPCR), four circRNAs were more highly expressed in healthy donors than in patients with BCP-ALL: circIL4R ($p < 0.0001$), circZCCH7 ($p = 0.0307$), and circX (intergenic) ($p = 0.076$). Furthermore, circPVT1 ($p = 0.0002$), circHIPK3 ($p < 0.0001$), circPAX5 ($p < 0.0001$), and circAFF3 ($p = 0.0115$) were overexpressed in patients with BCP-ALL. The expression of the target set of circRNAs according to BCP-ALL cytogenetic subtype showed circAFF2 was highly expressed in TCF3-PBX1 ($p = 0.0252$), BCP-ALL, and to a lesser extent in ETV6- RUNX1 BCP-ALL ($p = 0.021$). CircBCL2 (intronic) was upregulated in ETV6-RUNX1 fusions ($p = 0.0166$), circSETBP1 and circX (intergenic) were significantly reduced in MLL rearranged samples ($p = 0.0274$ and $p = 0.0472$, respectively), and circIKZF1 was lower in BCP-ABL and hyperdiploid leukemias than in the ETV6-RUNX1 subtype ($p = 0.0154$), in which the expression was conserved at levels comparable to B-cells. Overall, circRNAs have the potential to regulate specific cell functions, cell differentiation, maturation stages, and the growth of leukemic cells. As circRNA deregulation has been observed in patients with BCP-ALL, based on their cytogenetic subtype and previous cancers, circRNAs could potentially serve as markers for BCP-ALL. However, further research is necessary to determine the exact role that circRNAs play in leukemogenesis and their potential as markers for the disease [68][69]. **Figure 1** shows a summary of the biomarkers associated with BCP-ALL grouped as protein markers (**Figure 1**A), nucleic acid molecules (**Figure 1**B,C), metabolites (**Figure 1**D), and according to the different stages of disease.


A.
Usefulness
Biomarker A B C D E F G H I
A2M
Ac133
ACTB
ADA
ADAM-TS 15
Adiponectin
AFAM
AMBP
Anti-9-0AcSGs
APOA1
APOA4
APOE
APRIL
ATG3
BAFF
BCMA
C3
Ca125
CCD33
CD9
CD19
CD24
CD29
CD44
CD56
CD63
CD81
CD105
CD146
CD200
CERU
CFB
cKI-67
CLU
CLUS
CTAP-III
CTSD
Cytoplasmic actin
DAM17
EGFR
F2
FLJ26567
FURIN
GSTP
HLA-DR
HSP70
HSP90
IL-6
Leptin
LRG1
MPO
p53
Pf4
PGRN
PHB
PNPO
PRDX4
Proteasome ChT-L
Resistin
RPLP0
Semaphorin 4D
SERPINA1
SERPIND1
SERPINF2
sE-selectin
sFASL
sI-CAM-1
sL-selectin
Smad7
Statin
Survivin
sVCAM
SYND1
TACI
TGF-β1
Thymidine kinase
TM
TNF-R1
TNFRS2
TNFRS9
TNFRSF6B
TNF-α
TPI-1
TRAIL
B.
Usefulness
Biomarker A B C D E F G H I
Mitochondrial DNA
cf-DNA
circRNA-AFF2
circRNA-AFF3
circRNA-BCL2
circRNA-HIPX3
circRNA-IKZF1
circRNA-IL4R
circRNA-PAX5
circRNA-PVT1
circRNA-SETBP1
circRNA-X
circRNA-ZCCHC7
miR-100
miR-101
miR-10b
miR-125b
miR-125b-1
miR-125b-5p
miR-126
miR-128-3p
miR-128b
miR-129
miR-133b
miR-141
miR-142-3p
miR-146a
miR-146a-3p
miR-146b
miR-148a
miR-150
miR-155
miR-15b
miR-181a
miR-181b
miR-181b-5p
miR-181c
miR-193b
miR-196
miR-196a
miR-200c
miR-203
miR-20b
miR-21
miR-216
miR-217
miR-22
miR-221
miR-222
miR-223
miR-223-3p
miR-23a
C.
Usefulness
Biomarker A B C D E F G H I
miR-24
miR-25
miR-26a
miR-299-3p
miR-29a
miR-302b
miR-302d
miR-30c
miR-30e-3p
miR-325
miR-330
miR-335
miR-34a
miR-362
miR-368
miR-374
miR-383
miR-425-5p
miR-454
miR-494
miR-501
miR-511
miR-513
miR-514
miR-515-5p
miR-517b
miR-520
miR-532
miR-576
miR-585
miR-617
miR-625
miR-629
miR-638
miR-645
miR-696-5p
miR-9
miR-92a
miR-99a
mRNA Bcl-2
mRNA CRLF2
mRNA HLA-G
mRNA LEF-1
mRNA Livin
mRNA LRP
mRNA MS12
mRNA Survivin
mRNA-APRIL
mRNA-BAFF
mRNA-BCMA
mRNA-TACI
Overexpressed
Downexpressed
Variable
Unknown
A Diagnosis
B Differenciation betwen diseases
C Prognosis
D Risk stratification
E Response to treatment
F Mesurable Residual Disease
G Resistance to Therapy
H Target Therapy
I Relapse
D.
Usefulness
Biomarker A B C D E F G H I
11(Z),14(Z)-eicosadienoic Acid
1-Tetrahexanoyl-2-(8-[3]-ladderane-octanyl)-sn-GPEtn
2,3-Dinor-6-keto-PGF1a
3-Decaprenyl-4-hydroxybenzoic acid
8,11-eicosadienoic acid
Chenodeoxycholic acid glycine conjugate
DG (24:1(15Z)/22:5(4Z,7Z,10Z,13Z,16Z)/0:0)
FAD
GPCho(O-6:0/O-6:0)
GPEtn(16:0/0:0)
GPEtn(18:1(9Z)/0:0)
Hyaluronic Acid
LysoPC (15:0)
LysoPC (16:0)
LysoPC (17:0)
LysoPC (18:0)
LysoPC (18:1(11Z))
LysoPC (18:2(9Z,12Z))
LysoPC (20:5(5Z,8Z,11Z,14Z,17Z))
LysoPC (22:5(7Z,10Z,13Z,16Z,19Z))
LysoPC (22:6(4Z,7Z,10Z,13Z,16Z,19Z))
LysoPE (0:0/16:0)
LysoPE (18:1(11Z)/0:0)
LysoPE (20:4(8Z,11Z,14Z,17Z)/0:0)
LysoPE (22:4(7Z,10Z,13Z,16Z)/0:0)
Methyl 8-[2-(2-formyl-vinyl)-3-hydroxy-5--oxocyclopentyl]-octanoate
NADH
PA (22:0/0:0)

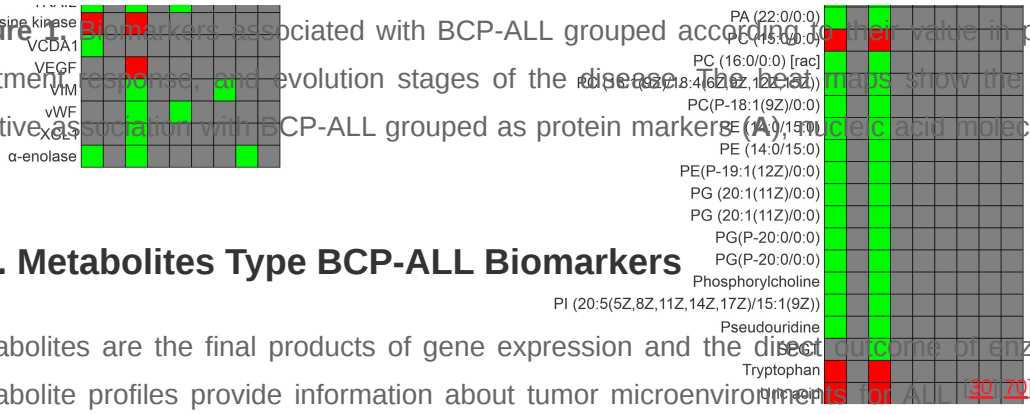


Figure 1. Biomarkers associated with BCP-ALL grouped according to their value in prognosis, risk stratification, treatment response, and evolution stages of the disease. The heat maps show the circulating biomarkers with positive association with BCP-ALL grouped as protein markers (**A**), nucleic acid molecules (**B**,**C**), and metabolites (**D**).

2.3. Metabolites Type BCP-ALL Biomarkers

Metabolites are the final products of gene expression and the direct outcome of enzymatic and protein activity. Metabolite profiles provide information about tumor microenvironments for ALL [30][70]. These metabolite profiles can be studied by metabolomics. “Metabolomics” involves the quantitative measurement of time of the metabolic responses of multicellular systems to pathophysiological stimuli or genetic modification [17]. Perturbation in the metabolism of patients at different disease stages could provide a unique metabolic signature to monitor treatment outcomes and disease progression [71]. Metabolism in cancer is a major research area in cancer biology. It examines how metabolic activities are changed in cancer cells compared to normal cells [30].

As shown in **Table 1** and **Figure 1**D, researchers have reported metabolic differences in patients with ALL compared to healthy controls. Musharraf et al. [70] compared the metabolite profiles in the serum of 96 patients with ALL, AML, and APA, obtained by using the nuclear magnetic resonance spectroscopy technique. The authors reported high lactate levels and low levels of alanine, glutamine, histidine, lysine, valine, and proline. They theorized that patients adopt a secondary metabolic pathway to generate glucose because cancer cells require more glucose than can be provided by glycolysis. These findings are in contrast with those reported by Morad et al. [30], who evaluated the plasma of patients with ALL (n = 14), AML (n = 16), and breast cancer (n = 25). They reported high threonine, proline, glycine, alanine and lysine levels and low lactate levels in the ALL group. These changes could be due to tumor tissue competing for nitrogen compounds found in the amino acid structure [30][70]. Another group found that fatty acids were elevated as reservoirs, corresponding with an accumulation of carnitine, which plays a role in fatty acid metabolism [70]. Upon closer analysis, there seem to be notable variations in the approaches employed, which could explain the discrepancies in the outcomes. A crucial factor to consider is that the patient selection was comprised of diverse cancer types, like breast cancer and aplastic anemia, which may have influenced the results. Furthermore, though both studies utilized nuclear magnetic resonance spectroscopy, the validation techniques differed. One study used variable importance in projection values, while the other used principal component analysis. To gain a comprehensive understanding of the implications of these disparities, providing further context and specific examples would be highly beneficial. Overall, with a more thoughtful and informed examination, people can better appreciate the nuances of these findings.

In a recent study focused on developing a novel photo-diagnostic strategy using fluorescence emission spectra, the authors used plasma or red blood cell extract from 45 patients with AL. They found four metabolites that were altered and correlated: nicotinamide adenine dinucleotide (NAD) + hydrogen (H) (NADH), FAD, tyrosine kinase, and tryptophan (p < 0.05). NADH and tryptophan were decreased in patients with ALL, and there was a connection between tyrosine kinase and tryptophan. However, FAD was increased in patients with AL. Thus, the AL diagnosis

can be made based on the FAD, NADH, tyrosine kinase, and tryptophan levels, but the ratios can help to discern between AML and ALL [8].

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