Epigenetic immune cell markers for Chronic Lymphatic Leukemia (CLL)

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Introduction

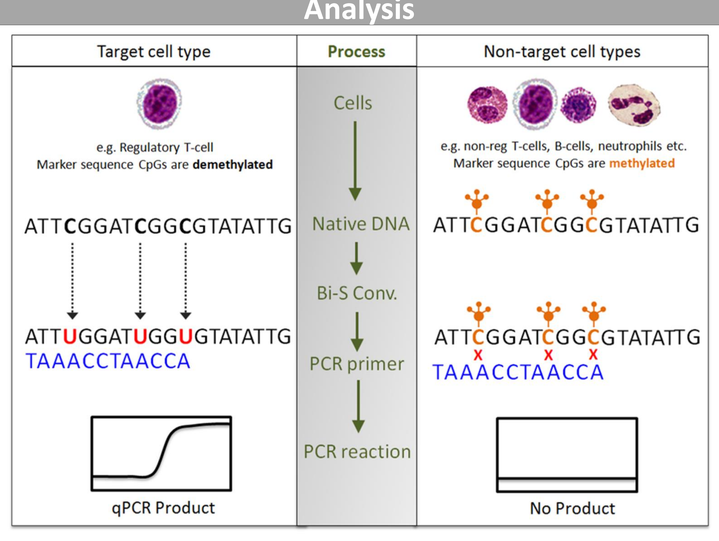
CLL is a rare hematological cancer classified as low-malignancy non-Hodgkin lymphoma. While rare, it is the most frequent form of leukemia in EU & US as it accounts for approximately a quarter of all leukemias. Since diagnosis is often incidential and late, this slowly advancing disease can develop into aggressive cancer. Earlier diagnosis may be the best tool to allow for timely intervention, better monitoring and longer survival.

Simple home tests revealing altered immune cell counts may provide a path towards better compliance with prophylactic screening for persons at higher age and thus higher risk of developing hematological cancers.

Capillary blood from finger- or heel-pricks collected on dried blood can be used for epigenetic immune cell counting and may provide hints for a conspicuous differential immune cell count.

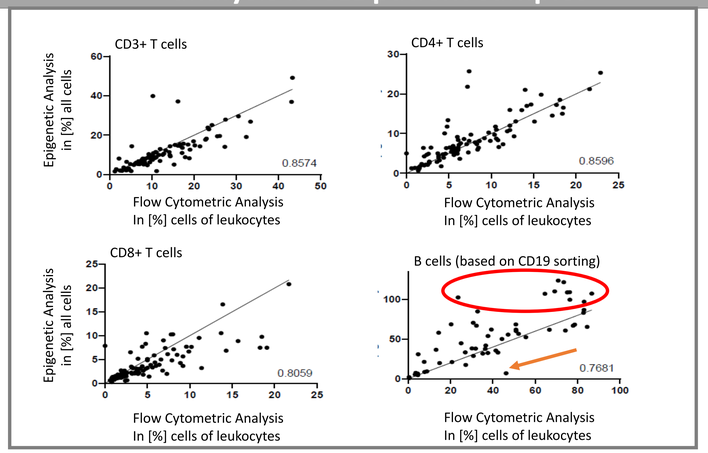
Here, we investigated if markers for various immune cell populations with a particular focus on B cells, obtained from blood of untreated patients, are indicative for untreated CLL.

Method: DNA methylation-based qPCR for Immune Cell Analysis



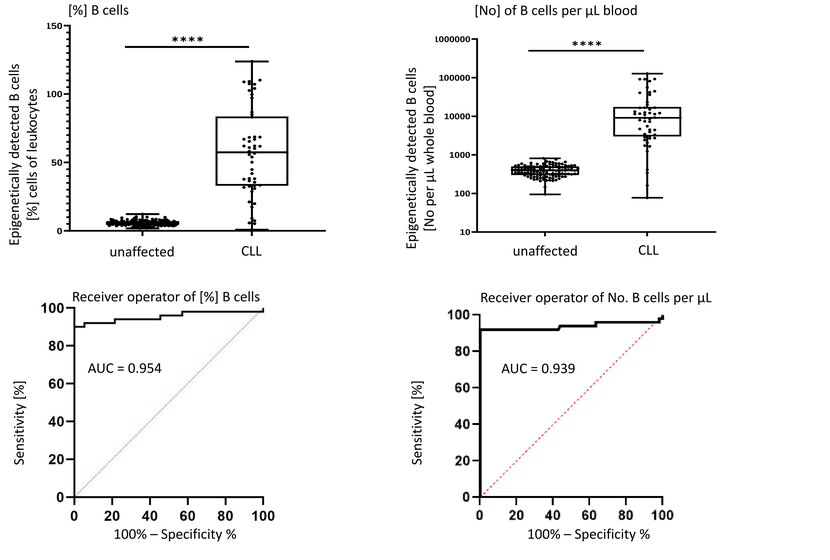
- DNA methylation is transferred into primary DNA sequence by bisulfite conversion
- Oligos match only the converted (demethylated) target sequence.
- The specifically unmethylated gene and the housekeeping gene GAPDH (or the methylated gene) are quantified by qPCR (1, 2).
- By counting the copy numbers of all GAPDH copies or the genes‘ methylated and unmethylated variant and the specifically unmethylated gene, the relative number of cells with this trait can be identified (and counted)

Epigenetic assays correlate well with flow cytometric cell analysis in CLL patient samples



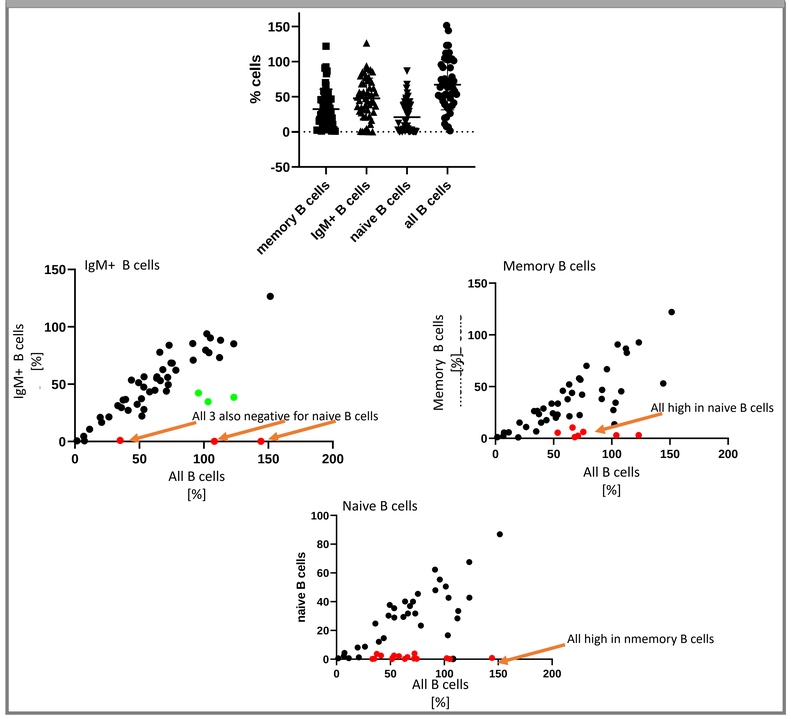
- When comparing flow cytometry for T cells with epigenetic qPCR, cell type quantification leads to substantial equivalence with Spearman coefficients above 0.8
- Expectedly, relative T cell counts are lower than expected for healthy individuals for both flow and epigenetic measurements
- B cell counts (CD19 for flow and LRP5 for qPCR) measure unphysiologically high independent of method.
- While correlation for B cell markers between technologies is also good, there are notable exceptions, e.g., one sample has cytometrically high B cell counts but is not observed as high in epigenetics (red arrow). Various blood samples measure high and partially “out of range” (appearing as above 100%, red circle) with epigenetics, but remain lower for flow.

Epigenetic counts for B cells accurately identify untreated CLL



- CLL patients show significantly higher B-cells in their peripheral blood than healthy donors.
- At a cut-off of 14.8% B cells, a 100% Specificity and 90% sensitivity is observed.
- At a cut off of 1026 B cells/µL a 92% sensitivity and a 100% specifity is reached.

Epigenetic markers for naïve, memory and IgM+ B-cell subpopulations



Conclusion

B cell markers, and in particular LRP5 as epigenetic marker for all B cells, appears to be a highly specific and sensitive marker for recognition of increased B cell numbers in patients with CLL.

For LRP5, this increase is visible both in absolute cell counts and in relative counts.

Markers for naïve, memory and IgM⁺ B cells are also unphysiologically high.

- With exception to 3 samples, all patients have high counts of IgM⁺ B cells indicating either incorrect or no class switch in most tumors.
- The epigenetic marker for naïve B cells subclassifies tumors as it is negative for a relatively high number of CLL samples likely stemming from switched B cells.
- Both, IgM and memory B cell markers are more similar to the general B cell (tumor) marker, but when negative then this fits to a naïve B cell phenotype clone.

Relative cell counts can be obtained very easily and accurately from dried blood spots, The epigenetic B cell markers appear to recognize B cell lymphocytosis – and partially subclassify this status.

The markers seem to lend themselves to unsupervised blood draw from capillary blood and thus be able to provide an efficient screening for B cell lymphocytosis.

Reference

[1]**Baron et al.**, Epigenetic immune cell counting in human blood samples for immunodiagnostics. Sci. Transl. Med. 2018 Aug; 10 –pp1-11

[2]**Wieczorek et al.,** Quantitative DNA methylation analysis of FOXP3 as a new method for counting regulatory T cells in peripheral blood and solid tissue. Cancer Res. 2009 Jan 15;69(2):599-608