Old data, new tricks:
Comprehensive computational analysis of 10 years of multi-center EuroFlow AML diagnosis data

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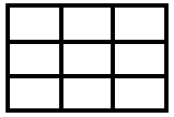
01.
Data

The dataset

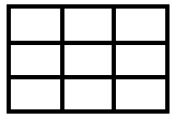
Patients with acute myeloid leukemia
EuroFlow ALOT + AML diagnosis panel
2011 – 2023
8 centers
5379 FCS files from 799 patients



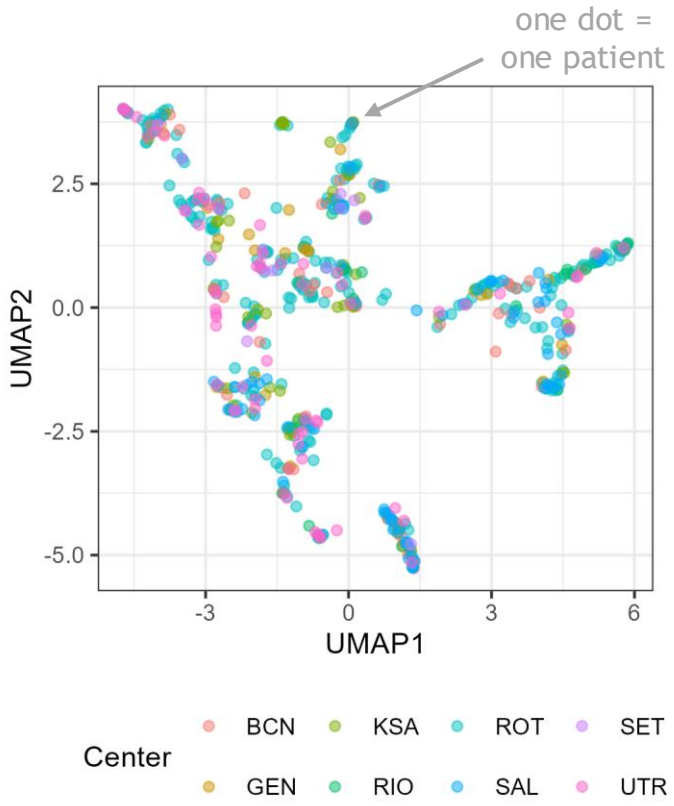
WHO 2008 classification

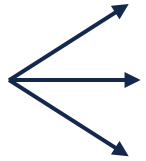
02.
Results

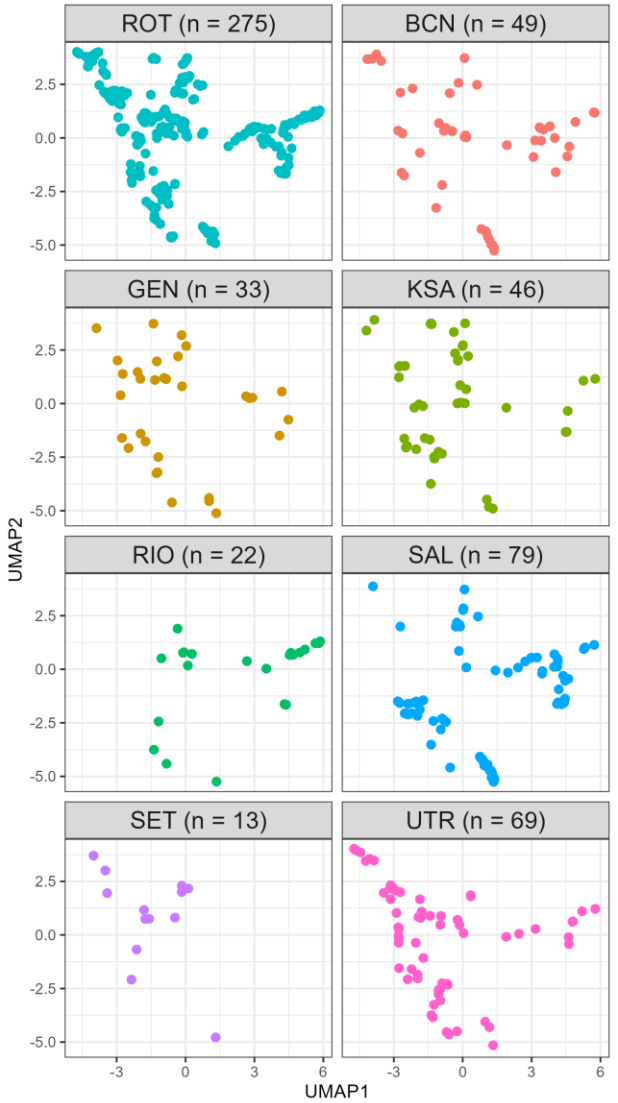
EuroFlow standardization mitigates center-specific batch effects



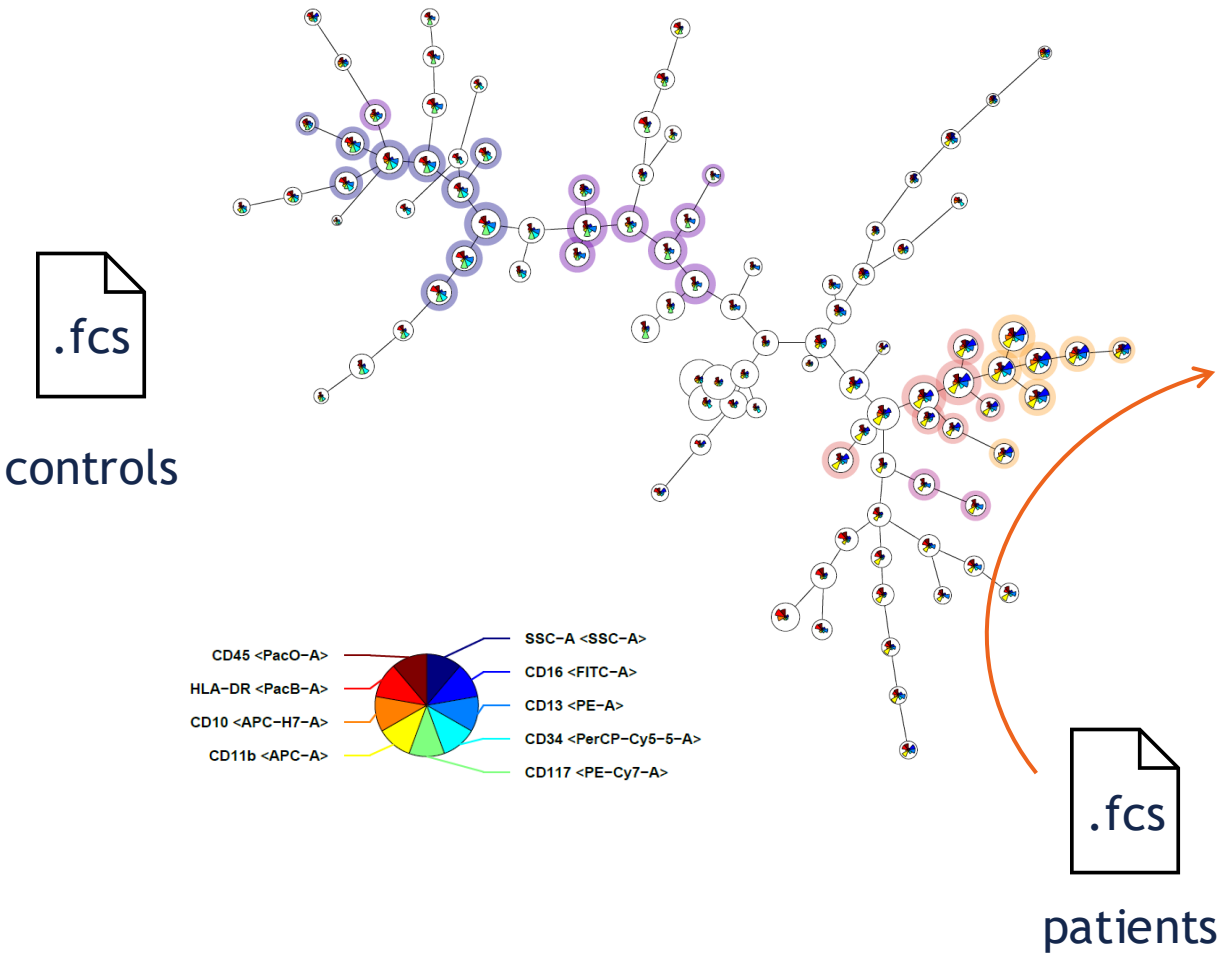
FlowSOM-derived cell population %



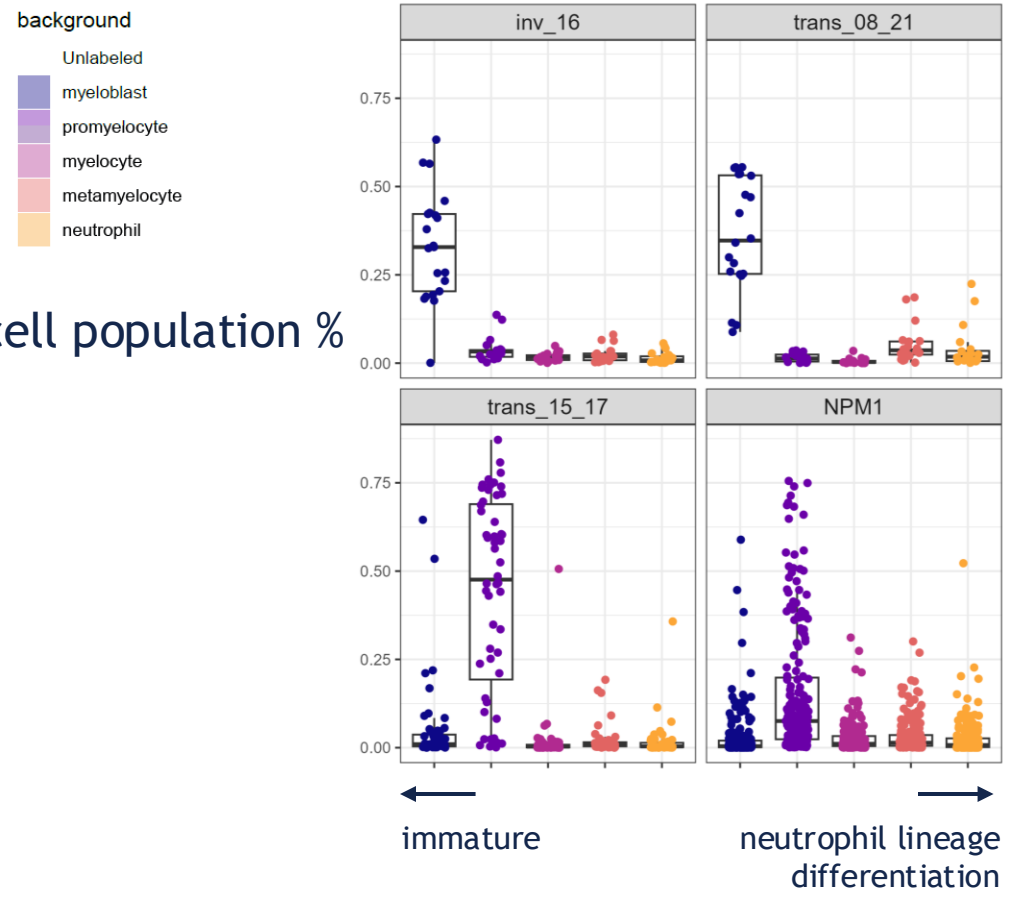




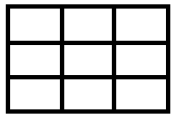
FlowSOM analysis locates maturation arrest at various stages for distinct WHO classes



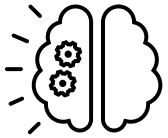
cell population %



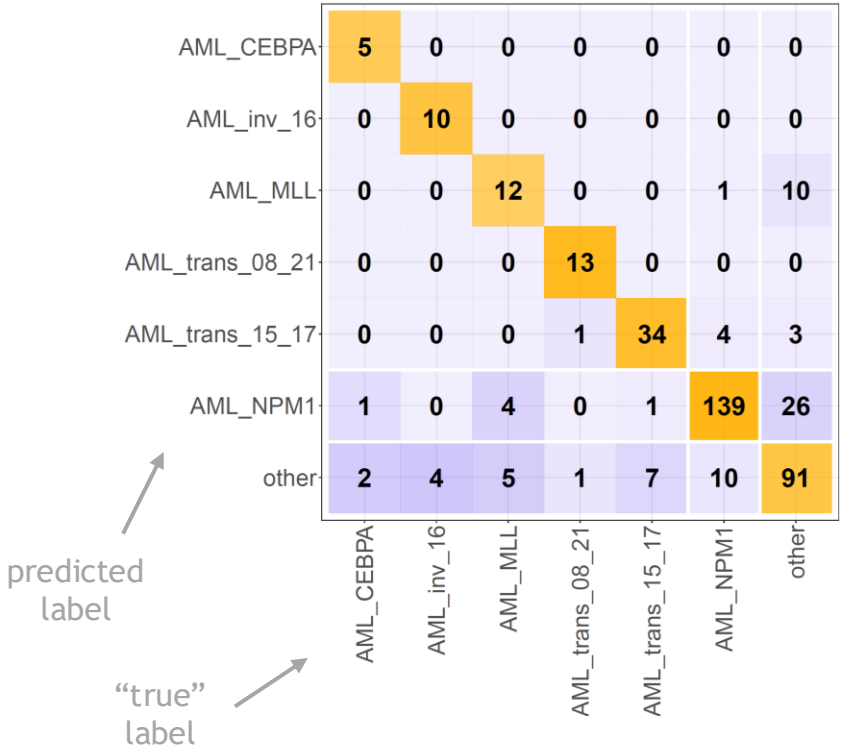
A random forest machine learning model can predict WHO genetic classes from immunophenotypic data



FlowSOM-derived cell population %



random forest machine learning model



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poster

Old data, new tricks: comprehensive computational analysis of 10 years of multi-center EuroFlow acute myeloid leukemia diagnosis data

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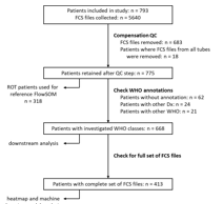
Acute myeloid leukemia (AML) is a malignancy with high inter- and intra-patient heterogeneity, both at the genotypic and phenotypic level, posing challenges in diagnosis, prognosis and treatment. The most recent edition of the WHO classification divides AML into AML with defining genetic aberrancies and AML defined by differentiation. Here, we investigated AML immunophenotypes and WHO classes, with a comprehensive computational analysis on an extensive AML dataset.

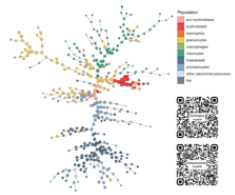
Data & data analysis

File selection



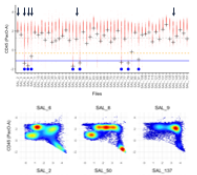
Reference FlowSOM

One FlowSOM model was created per tube of the EuroFlow AML panel, with non-**lymphocyte** cells from one center (large cohort, both pediatric and adult)



Compensation QC

Per center and per marker:
Files were removed if
q5 < median(q5) - 2 * MAD(q5)



Random forest model

Metacluster percentages (MC%) were used as input features. Within a 5-fold cross-validation, Synthetic Minority Oversampling Technique (SMOTE) was used to overcome WHO class imbalance.

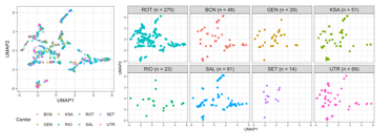
First, one RF model using the complete training dataset was used to determine feature importance. Features with correlation higher than 0.4 were removed, resulting in 119 from a total of 358 features being used for training of the model.

RF model predictions result in a prediction score for each WHO class. The class with the highest score was assigned as the predicted WHO class.

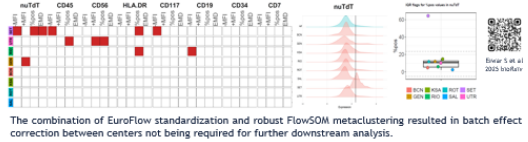
Results

EuroFlow standardization mitigates center-specific batch effects

UMAP with FlowSOM-derived metacluster % (tube 4)



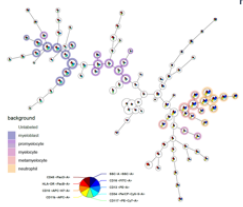
CytoBatchFlagR framework for batch effect detection (results for tube 4 of the panel)



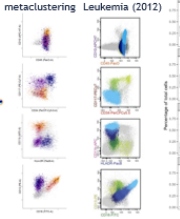
The combination of EuroFlow standardization and robust FlowSOM metaclustering resulted in batch effect correction between centers not being required for further downstream analysis.

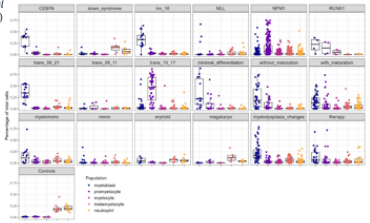
FlowSOM analysis locates maturation arrest at various stages for distinct WHO classes

FlowSOM based on 10 healthy controls



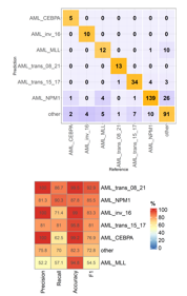
FlowSOM-based van Dongen *et al* metaclustering Leukemia (2012)



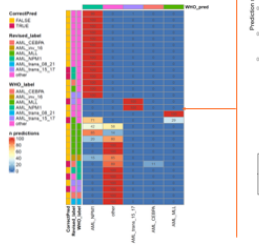


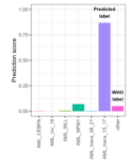
With FlowSOM we were able to identify neutrophil maturation stages (tube 1). Some genetic alterations lead to a maturation arrest at earlier stages. Patients from WHO classes like inv(16) and t(8;21) have more immature cells, whereas cells from other patient groups like *NPM1*mut and t(15;17) are more differentiated towards the neutrophil lineage. For the WHO diagnostic groups defined by differentiation, labels corresponded to the observed maturation arrest.

A random forest machine learning model can predict WHO genetic classes from immunophenotypic data

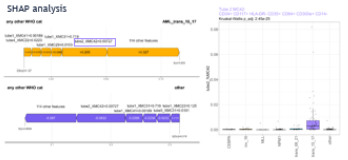


When performing 100 iterations of the RF model, 31 patients were wrongly predicted in each iteration. We investigated these in-depth and doublechecked their assigned WHO labels.

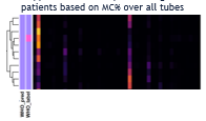




SHAP analysis



snippet from heatmap clustering all patients based on MC% over all tubes



The random forest model could accurately predict several genetic abnormalities, and these results were reproducible in an independent validation cohort (data not shown). In addition, some of the mispredictions hinted to actual mislabeling, or missing information, from the metadata.

When training machine learning models for clinical use, it is imperative to use correct metadata labels in the training dataset.

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