Epigenetic Immunophenotyping in Monitoring of SARS-CoV-2 Vaccine Response and COVID-19 Disease Course

Janine Jung[1], Ulrich Hoffmueller[1], Björn Samans[1], Eva Raschke[1], Marta Rossello Chornet[2], Konstantin Schildknecht[1], Laura Lozza[1], Araceli Rossello Chornet[1], Lourdes Alos Zaragoza[2], Javier Hernandez Laforet[2], Nina Babel[3], Sven Olek[1]

[1] Ivana Türbachova Laboratory for Epigenetics, Epiontis, Precision for Medicine GmbH, Berlin, Germany; [2] University General Hospital of Valencia, Spain; [3] University Hospitals Ruhr-University Bochum, Germany

Objective

The objective was to explore whether epigenetic immune cell counting can advance efficiency and quality of diagnostic and immune monitoring related to COVID-19. Application areas were monitoring disease course, therapeutic clinical development, and measurements of SARS-CoV-2 vaccine responses.

Method

Immune cell type specific epigenetic assays have been developed over the last decade. They are primarily used in therapeutic clinical research in oncology and autoimmune disease. Due to the high sample stability and low amount requirements for epigenetic measurements and available assay portfolio for key immune cell populations relevant in COVID-19, the method promised to be useful and practical in the pandemic setting.

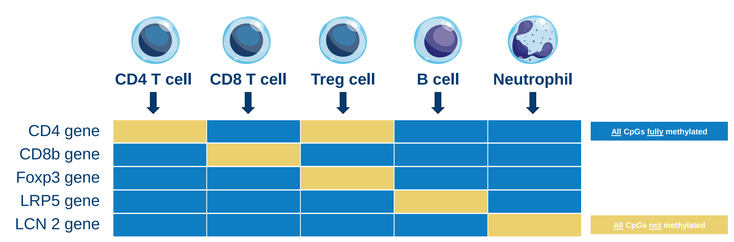
Application in COVID-19 Disease Course (A)

Epigenetic immunophenotyping using whole blood of hospitalized COVID-19 patients was applied and CD3, CD4, CD8 and regulatory T cell populations, NK cells, naïve and memory B cells were quantified, and measurement results show to predict mild or severe COVID-19 disease courses. Furthermore, nasopharyngeal swab and saliva samples were applied demonstrating that epigenetic immune monitoring can measure immune cell content in such non-invasive sample types. Due to the low sample volume and handling requirements and availability of 35 assays for relevant immune cell populations, epigenetic immune monitoring is suitable for therapeutic COVID-19 clinical trials.

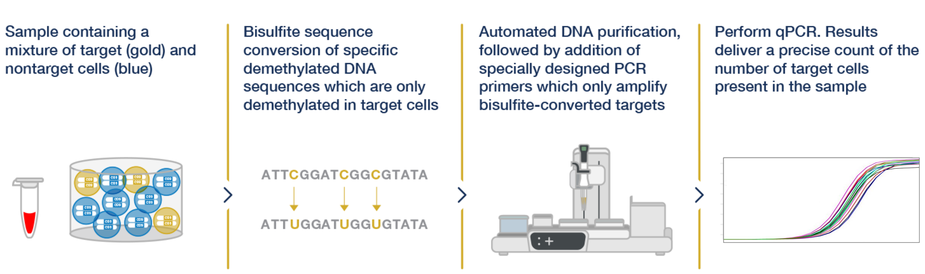
Application in Monitoring SARS-CoV-2 Vaccine Response (B)

Another possibility for sample collection is single drops of capillary blood deposited on filter paper (dried blood spots), which have been collected pre and post SARS-CoV-2 booster vaccinations in healthy subjects. Such measurements revealed vaccine response for example in changes of cell populations epigenetically active in the markers CCR7 and TIGIT.

Epigenetic Cell Counting – Schematic:



Epigenetic Cell Counting – Measurement Process:



A: Epigenetic Immune Monitoring in patients

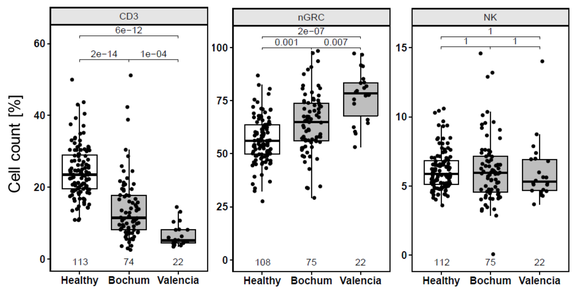
- Peripheral blood samples from unvaccinated, hospitalized COVID-19 patients were collected at Hospitals in Bochum (Germany) and Valencia (Spain)
- Disease stage was assigned according to Robert-Koch-Institute classification
- 113 pre-pandemic Caucasian healthy donor (18-71yrs.) samples were collected and purchased from in.vent GmbH (Germany)

		Disease stage (initial visit) n=			
Cohort	Patients with available first visit n=	Mild/Asymptomatic/ Moderate	Severe	Critical	Unkown
Bochum	75	42	20	8	5
Valencia	22	/	21	1	/

Patients were grouped based on their initial and the next reported visit as:

- "poor prognosis": Change from moderate to severe or critical OR severe to critical
- "good prognosis": Change from severe or critical to moderate OR critical to severe or moderate OR stably moderate

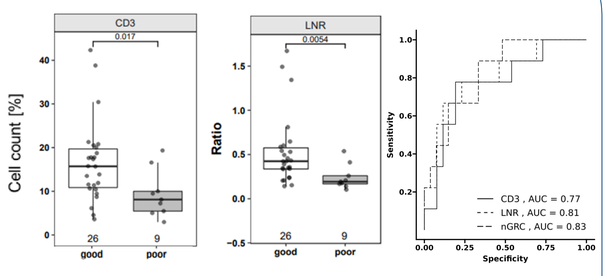
A: Disease and Site Dependently Decreased T Cells and Increased Neutrophils



Comparison of lymphocyte populations of healthy and COVID-19 case cohort:

- Disease cohorts have different cell counts due to different stages at inclusion
- Significantly lower CD3⁺ T cell count in patients
- Significantly higher Neutrophils in patients
- No significant difference in NK cell counts in patients
- All p values (adjusted according to Bonferroni correction) relate to the Wilcoxon rank sum test for median differences, analysis performed for each assay separately

A: CD3 and LNR as Strong Prognostic Markers for Disease Outcome

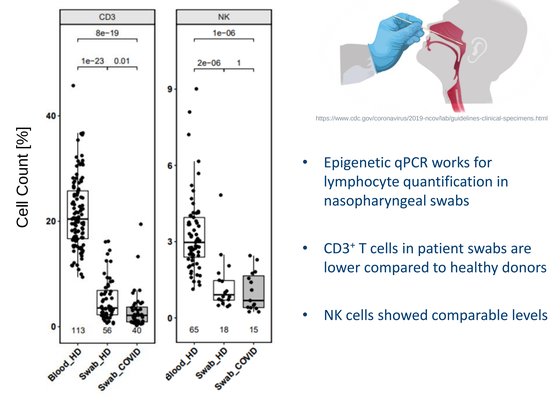


Evaluation of the relation between clinical course and immune cell values at visit 1:

- Both a higher CD3⁺ T cell count and a higher LNR (lymphocyte-to-neutrophil ratio) correlated with favorable clinical outcome

Marker	AUC (95% CI)	Specificity	Sensitivity	Accuracy	Optimal Threshold
T cells	0.77 (0.59-0.96)	0.81	0.78	0.80	10.2% cells
Neutrophils	0.83 (0.68-0.97)	0.67	0.89	0.72	58.6% cells
LNR	0.81 (0.63-0.98)	0.88	0.67	0.83	0.21

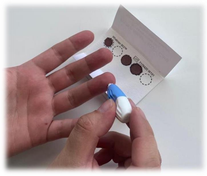
A: Low CD3⁺ T Cells in Nasopharyngeal Swabs in Patients



- Epigenetic qPCR works for lymphocyte quantification in nasopharyngeal swabs
- CD3⁺ T cells in patient swabs are lower compared to healthy donors
- NK cells showed comparable levels

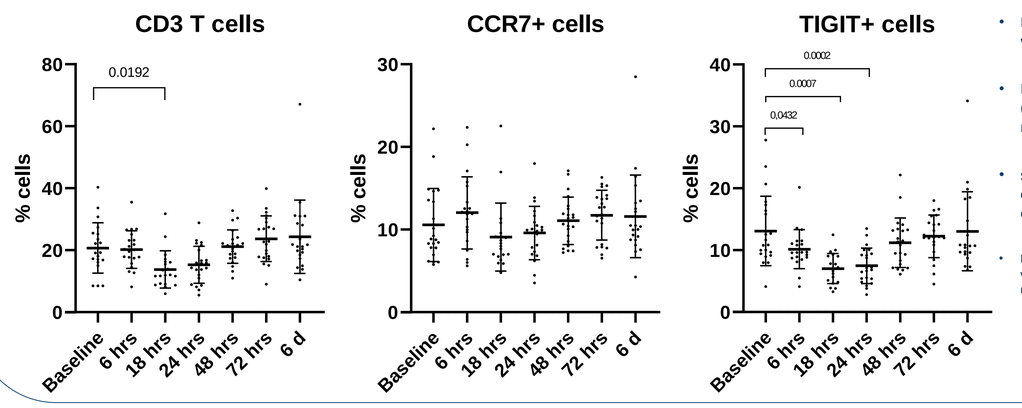
Blood_HD: Pre-pandemic healthy donors
Swab_HD: Nasopharyngeal swabs from healthy donors
Swab_COVID: Nasopharyngeal swabs from COVID-19 patients

B: Epigenetic qPCR Analysis of T cell Response After Third COVID-19 Vaccination



- Dried blood spots (DBS) from 22 healthy donors (15 female, 7 male, Age 24-50) who received the third COVID-19 vaccination
- Samples from before and at various time points after vaccination (6, 18, 24, 46 hours and 6 days)
- DBS sample collecting can be performed by untrained individuals in a point-of-care or home setting
- Epigenetic qPCR analysis of CD3, CCR7, CTLA4, TIGIT, B cells, memory B cells, and IgM⁺ B cells
- Shown are the results for CD3⁺ T cells, CCR7⁺ and TIGIT⁺ cells

B: Decrease of CD3⁺ T cells After Vaccination



- Decrease in CD3⁺ T cells within 6-48 h
- Less decrease in CCR7⁺ cells (Assay monitors the migration marker CCR7)
- strongest reduction in TIGIT⁺ cells (Assay monitors the exhaustion marker TIGIT)
- P values shown have been calculated versus baseline with Dunnett's multiple comparisons test

Conclusion

A:

- Reduction of CD3⁺ T cells in whole blood and in the nasopharynx, No differences for NK cells
- High CD3 count and higher LNR correlated positively with favorable clinical outcome
- T cells, neutrophils and LNR are prognostic for disease outcome

B:

- Significant decrease of T cell subsets after booster (3rd) vaccination
- Epigenetic qPCR indicates T cell response 6h after vaccination

Epigenetic cell type determination allows lymphocyte quantification from a wide range of sample matrices, including DBS and nasopharyngeal swabs

Epigenetic qPCR enables unsupervised home-based immune monitoring