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**Topics:** A

**Titel:** *“Does complement sensitization of red blood cells influence the osmotic fragility assay”*

**Authors**

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**Abstract**

**Introduction**

The Department of Clinical Immunology at Rigshospitalet, Copenhagen University Hospital use flow cytometry to investigate red blood cell (RBC) properties, e.g. complement binding to RBCs and osmotic fragility (OF) assay. The OF assay reveals how fragile RBCs are in a series of hypotonic solutions of decreasing saline concentration. The assay is used to help diagnose patient cases with, for example, hereditary spherocytosis or xerocytosis.

Initial trials of the OF assay has revealed a possible interaction between RBC complement sensitization and OF.

In this study we made a 95% reference interval for the OF assay and investigated the influence of complement sensitization on RBCs in the OF analysis.

**Methods**

For the OF analysis a phosphate buffered saline solution (PBS) with a conductivity of 17.3-17.8 mS/cm was used. PBS was used undiluted (100% PBS) or diluted in distilled water to give PBS buffer solutions of 60%, 50%, 40% and 30% PBS.

A countdown of 3 minutes was started and 2 µl washed, packed RBC was suspended in 100% PBS. After 30 seconds the next suspension was made with the 60% buffer solution. This was done repeatedly until all buffer suspensions was made and with 30 seconds left, acquisition was started on the flow cytometer. 10.000 events were collected using a Canto flow cytometer from BD and the percentage of intact RBC was calculated using a FSC/SSC scatterplot.

129 blood samples, from Danish blood donors were used to establish a reference interval. The reference interval was defined as the interval between the 2.5 percentile and the 97.5 percentile.

Two patient cases with complement bound to the RBC were included in the study.

SPSS version 22 and Excel 2010 were used to analyze the data.

## **Results**

Reference interval for OF:

100% PBS: 96.2%-99.9%

60% PBS: 96.5%-99.8%

50% PBS: 49.4%-96.4%

40% PBS: 1.1%-22.4%

30% PBS: 0.0%-1.5%.

Two patients with complement bound to the RBCs, also had a decreased osmotic fragility at 40% PBS concentration. A percentage of 58% and 48% intact cell was found compared to the 95% reference interval of 1.1-22.2%. At 30% PBS concentration a similar decrease was found; 8.6% and 22.2% intact cells compared to the 95% reference interval of 0.0-1.5%.

## **Conclusion**

A 95% reference interval for the OF analysis from 129 blood samples was established.

The data presented in this abstract, indicates that there is an interaction between complement bound to RBCs and osmotic fragility. More patient cases are needed to verify this connection.