Image-based modelling, identification and counting of true Howell-Jolly Bodies in human erythrocytes

Oguzhan Angay¹, Mike Friedrich¹, Jürgen Pinnecker¹, Klaus Hempel², Katrin G. Heinze¹*

¹Rudolf Virchow Center for Experimental Biomedicine, University of Würzburg, Josef-Schneider-Str.2, 97080 Würzburg, Germany
²Department of Nuclear Medicine, University of Wuerzburg, Oberduerrbacher Strasse 6, D-97080, Würzburg, Germany
E-mail: oguzhan.angay@virchow.uni-wuerzburg.de

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The spleen selectively removes cells with intracellular inclusions e.g. clusters of DNA in circulating erythrocytes, called Howell Jolly Bodies (HJBs) [1]. With absent splenic function, HJBs appear in the peripheral smear and can be used as a simple and non-invasive risk-indicator for severe (bacterial) infections [2]. However, it is still under debate whether their counting is a reliable measure of splenic function. One reason is the risk of false positive results by unspecific nuclear remnants in the prepared smear samples that do not originate from HJBs, but from cell debris residing above or below the cell. Here, we present a method to improve accuracy of image-based smear sample tests that can be performed even in non-specialized medical institutions. We show how to selectively label HJB-like clusters in human blood samples and how to only count those that are undoubtedly inside the cell. We found a ‘critical distance’ referring to a relative HJB-Cell distance that true HJBs do not overshoot. To rule out false positive counts we present a simple inside-outside-rule based on this critical normalized HJB-Cell distance—a robust parameter that can be easily assessed by combining conventional 2D imaging and solely freeware programs for image analysis. Besides data based on fluorescence imaging, simulations of randomly distributed HJB-like objects on realistically modelled cell objects demonstrate the risk and impact of biased counting in conventional analysis.

REFERENCES: