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Developing normal reference ranges for dihydrorhodamine (DHR) test using whole blood (retrospective analysis)

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Abstract

Introduction: Chronic granulomatous disease (CGD) is an inherited primary immunodeficiency disease that results from a defect in one of the respiratory burst oxidase (NADPH oxidase) genes that form its components. The diagnostic laboratory assessment for CGD includes evaluation of NADPH oxidase function in neutrophils, using the more analytically sensitive DHR test.

Aim: The main objective of this project is to develop an internal reference range by determining the relative proportion of oxidizing cells percentages and the mean fluorescence intensity (MFI) in which both will provide a complete interpretation of the test results and making the result more accurate and reliable.

Methodology: A retrospective analysis was conducted from 107 individuals who were referred to the DHR test from IPPT and HUSM. We analysed data using FlowJo and SPSS. A logistic regression test was used to determine the relationship between the study groups with the test parameters. Logistic regression and Receiver Operating Characteristic (ROC) analysis was used to estimate the precision of the DHR-test.

Result: According to our findings the optimum cut-point for IPPT to differentiate CGD patients from healthy with fMLP% was 0.26%, and FMLP MFI was 9.15. While PMA% cut-point was 58.70% and PMA MFI cut-point was 50.00. On the other hand, according to HUSM data, the best possible cut-point for fMLP% was 3.86%, and FMLP MFI was 999.50. Whilst PMA% cut-point was 45.69% and PMA MFI was 1130.50. Moreover, AUC of the parameters were also high.

Conclusion: This study confirmed that developing an internal reference range is important for the accurate diagnosis of CGD. This data showed that there was a difference between the two centres in terms of test results and cut point.