

PATOLOGIE DELL'EMOSTASI PRIMARIA E PIASTRINOPENIE

## A COMBINATION OF TWO RAPID IMMUNOASSAYS IMPROVES HIT DIAGNOSIS.

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### Background and Aim:

Heparin induced thrombocytopenia (HIT) is an immune adverse reaction induced by the exposure to heparin which leads to the production of IgG antibodies against heparin-platelet factor 4 (H-PF4) complexes. The diagnosis is suspected based on a clinical evaluation with the 4T pre-test probability score (4Tscore) and required the detection of circulating antibodies bound to H/PF4 complexes by immunological and functional assay. Immunological assays are easy to perform, widely available and show high sensitivity. Functional assays are the gold standard for HIT diagnosis after a positive immunological test. However functional tests are technically demanding, time-consuming and limited to few, expert laboratories. This study aims to evaluate the performance of a combination of two automated rapid immunoassays -LIA and CLIA -with respect to the gold standard: an immunoassay -CLIA- followed by functional test -HIPA- for HIT diagnosis.

### Methods:

This observational retrospective study investigated 100 patients from Azienda Ospedaliera Careggi and other hospitals across or outside Tuscany, for whom clinicians requested H-PF4 antibody detection for HIT suspicion. Citrate whole blood and serum were collected and all samples analysed by CLIA assay at the time of sample submission. Then, we performed HIPA test, LIA and CLIA test on ACL TOP 970 on all 100 samples.

### Results:

CLIA test: 68 patients out of 100 (68%) were negative (<1U/mL) and 32 (32%) were positive ( $\geq$ 1U/mL). As CLIA test was performed by both Acustar™ system and ACL TOP 970 CL, we found concordant results. The functional test con-

firmed HIT diagnosis in 26/32 patients and excluded HIT in 6/32 patients. Based on these results, CLIA method showed a sensitivity of 93.30% and a specificity of 91.78%, a Positive Predictive Value (PPV) of 81.25% and a Negative Predictive Value (NPV) of 98.53%.

LIA test: 64 out of 100 patients (64%) were negative (<1U/mL) and the remaining 36 (36%) were positive ( $\geq$ 1U/mL). The HIPA tested 24/36 positive patients and 12/36 negative patients; only 3/64 LIA negative patients were diagnosed as HIPA positive while 61 patients were confirmed as negative. LIA test displayed a sensitivity of 88.89% and specificity of 83.56%, a PPV of 66.67% and NPV of 95.31%.

HIPA functional test was performed on all 100 patients and confirmed HIT diagnosis on a total of 27 patients (27%), 23 patients were positive on both CLIA and LIA test, 1 patient was negative on CLIA and positive on LIA test while 3 patients resulted positive on CLIA and negative on LIA.

By combining CLIA and LIA methods, a sensitivity of 100% and a specificity of 82.2% have been achieved. The PPV was 67.5% and NPV 87%. Among the 100 patients, the combination of CLIA and LIA tests allowed us to categorize 27 true positive, 13 false positive, 0 false negative and 60 true negative patients.

### Conclusion:

Based on our results, the combination of CLIA and LIA test improves the diagnosis of HIT by identifying all HIT positive patients and achieving a 100% sensitivity compared to single CLIA and LIA tests. Our study demonstrates how combined LIA/CLIA testing optimizes diagnostic sensitivity, with progressively greater probability of detecting platelet-activating antibodies with higher assay reactivity that reaches 100% when both automated assays yield moderate or strong results.

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