Platelet ATP release measurement. Whole blood or platelet rich plasma?

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Introduction:

Whilst light transmission aggregometry has been accepted as the 'gold standard' method of evaluating potential platelet dysfunction for many years, the technique does not always identify defects of platelet dense granule secretion.

Luciferase enzyme may be used to measure release of ATP from dense granules of activated platelets. The Chronolog® analyser (Chrono-log corporation, Havertown, PA, USA) allows the release of ATP from activated platelets to be measured in real time in addition to performing aggregometry studies.

Our laboratory has utilised the system to measure ATP in whole blood (WB) and screen for platelet secretion defects. Platelet nucleotide analysis is used as a confirmatory test.

WB has been used as the reduced sample volume was considered advantageous for paediatric evaluation. Review of process suggested that greater efficiency would be gained by the use of platelet rich plasma for all analyses.

A cross verification was performed to compare ATP release by luminescence using WB and platelet rich plasma (PRP) from matched samples.

Methods

27 samples were analysed for ATP release from platelets in response to 5µg/mL collagen agonist in both WB and PRP preparations. Collagen agonist targets the GPVI and α2β1 platelet receptors. Luminescence generated in response to 2nM ATP was established as a reference in each sample type. The ATP release following collagen was adjusted for the platelet count obtained for each sample and the results statistically compared.

Fig 1: Chrono-Log Model 700 Whole Blood Lumi-aggregometer

Method measuring Collagen using Whole Blood

Equal volumes of saline and whole blood from patient and a volunteer donor control were warmed to 37°C in stirred cuvettes after which time chronolume luciferase reagent was added to each.

Following incubation 2nM ATP standard was added and the instrument gain set. This setting allows the measurement of ATP release from the sample in response to specific platelet agonists.

Saline and whole blood from patient and donor was again mixed, warmed and luciferase added. The ATP release from 5µg/mL collagen was measured using the gain previously set.

The results were adjusted for platelet count to standardise and compare to an in house reference range.

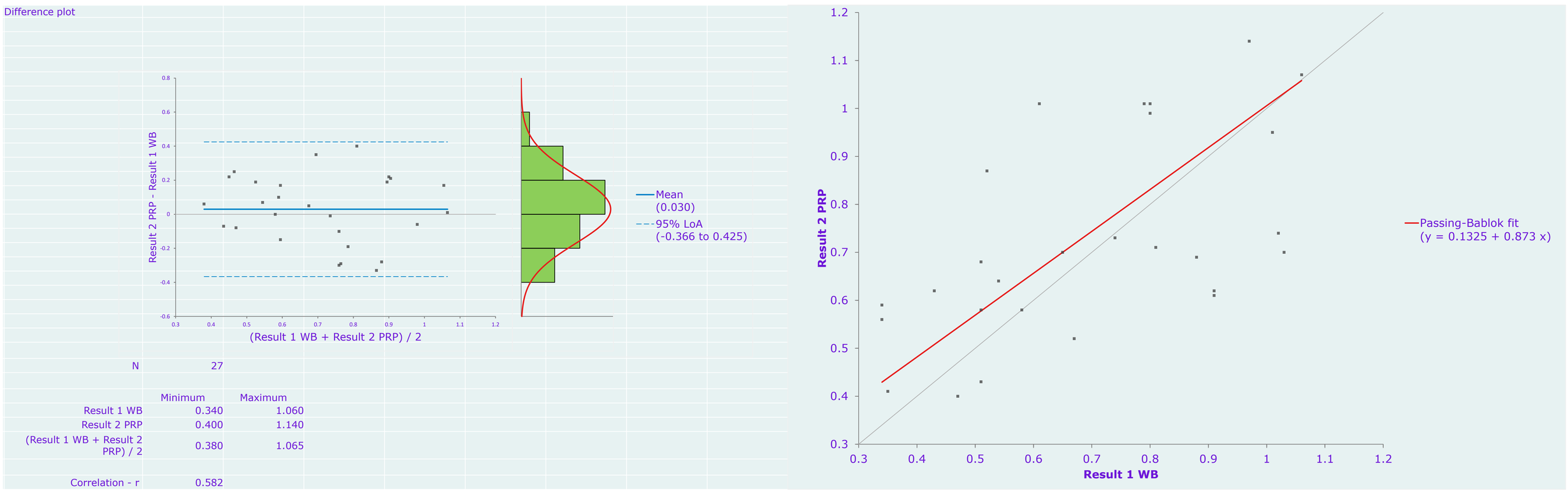
Method measuring Collagen using Platelet Rich Plasma

Platelet rich (PRP) and platelet poor plasma (PPP) were prepared by centrifugation. PPP was added to a cuvette and placed in the appropriate well as a blank.

PRP was added to another stirred cuvette and warmed to 37°C. Chronolume reagent was added and incubated followed by ATP standard as before. Again the instrument gain was set using 2nM ATP for both patient and donor control PRP.

The process was repeated and ATP release in response to 5µg/mL collagen measured. The ATP release was adjusted for PRP platelet count.

Paired ATP release results obtained from both patient and controls in response to collagen were evaluated by Bland Altman and Passing Bablok analysis using Analyse-It® for Microsoft excel software.



	Minimum	Maximum
N	27	
Result 1 WB	0.340	1.060
Result 2 PRP	0.400	1.140
(Result 1 WB + Result 2 PRP) / 2	0.380	1.065
Correlation - r	0.582	

Fig 2.0 Method comparison: Result 1 WB, Result 2 PRP

Results

Mean values for ATP release were 0.70 and 0.71 nM for WB and PRP respectively. Bland Altman analysis showed mean difference of 0.03 nM for PRP overall with limits of agreement between -0.366 and 0.425. Correlation was adequate with an r^2 value of 0.582.

Conclusion

The analysis shows that the ATP release measurement from platelets using the chronolog analyser showed acceptable consistency between values obtained using WB and PRP once the results are corrected for the platelet counts.

References

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