Thymosin beta-4 as a plasma biomarker?
Watch out for residual platelets!
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Centre for Life Sciences (CeLS), Level 1

Introduction
There would be three components in my seminar presentation: our recent publication on evaluating thymosin beta-4 (TB4) as a heart failure biomarker (Drum et al., 2017), my subsequent follow-up works that revealed residual platelets as a confounder of plasma TB4 measurements, and a proposal to overcome problems associated with residual platelets in biomarker screening. This seminar would be of upmost interest to fellow researchers in the field of blood biomarker discovery.

Thymosin beta-4 as a Potential Heart Failure Biomarker
Thymosin beta-4 (TB4) is a cardioprotective and angiogenic peptide. It is hypothesised that endogenous TB4 levels would change during cardiovascular disease as a compensatory response, and hence could be a useful biomarker. To evaluate TB4 as a potential heart failure biomarker, we quantified TB4 in plasma samples from the Singapore Heart Outcome and Phenotype (SHOP) cohort. Interestingly, TB4 turns out to be a female-specific biomarker in heart failure: TB4 is elevated in plasma of female heart failure with preserved ejection fraction patients, correlates with female all-cause mortality, and correlates well with other X-linked and sex-hormone regulated biomarkers. Circulating TB4 seems to be involved in the pathophysiology of female heart diseases.

Residual Platelets Could Confound Plasma TB4 Measurements
While doing further investigations into plasma TB4, I made a seemingly absurd observation: When blood samples were spun on the centrifuge for longer duration or at higher g-force, TB4 readings in the resulting plasma would drastically fall. At centrifugation speed of 200g for 10 minutes, TB4 readings in EDTA plasma were elevated (>1000 ng/ml) compared to when centrifuged at 2000g for 10 minutes (<100 ng/ml). Platelet counts were ~35×10^3/ml and undetectable respectively, strongly suggesting that residual platelets were contributing to plasma TB4 readings. Degradation of TB4 was eliminated as a possibility, because resuspending the pellet would almost completely restore the initial high TB4 readings.

I began to do a little “investigative journalism” to verify my hunch. In the literature, there are a total of 17 clinical studies measuring TB4 in healthy controls, 12 in serum and five were in plasma. I disregarded studies in serum because the platelet coagulation process is known to elevate TB4 readings as well. Of the five studies in plasma, only three studies provided information on centrifugation parameters—consistent with my hunch, median TB4 readings in controls is negatively correlated with centrifugation speed.

A Proposed Tool to Detect and Correct for Residual Platelets in Plasma
This realization opened a can of worms: how many plasma/serum biomarker screening studies out there were just comparing differences in platelets? In the coming months, I would be systematically cataloguing the analyte content of platelets from literature and from our own -omics screening. This would form a list of analytes that fellow blood biomarker hunters should check against. We also hope to find an invariant (or “housekeeping”) analyte that reliably correlates with platelet content in plasma at the point of freezing, thus allowing for post-hoc platelet normalisation and correction of biomarkers measurements in plasma biobanks.