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Decreased Calcium Content and $^{45}Ca^{2+}$ Uptake in Down's Syndrome Blood Platelets

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Summary

Total intracellular Ca^{2+} and $^{45}Ca^{2+}$ uptake has been studied in blood platelets from subjects with Down's syndrome and matched controls. In Down's subjects, Ca^{2+} levels (85.5 ± 5.9 nmol/10^{-9} platelets) were significantly lower than controls, 174 ± 10.0 nmol/10^{-9} ($p < 0.0005$). A similar reduction was seen in calcium uptake (Down's platelets, 0.79 ± 0.06 nmol/10^{-9} platelets; controls, 1.17 ± 0.07 nmol/10^{-9} platelets, $p < 0.005$). The low levels of intracellular Ca^{2+} may be related to decreased granular storage of serotonin, and the decreased Ca^{2+} uptake with impaired transport by intracellular Ca^{2+}-accumulating organelles such as the dense tubular system.

Abbreviation

PRP, platelet-rich plasma

Calcium ions play an essential role in initiating or terminating cellular functions by mechanisms that either involve the influx of Ca^{2+} or the intracellular translocation of Ca^{2+} (11). In blood platelets, Ca^{2+} content can be described as a three-compartment space, a surface component, and two intracellular components, one rapidly exchangeable with external Ca^{2+} and the other virtually nonexchangeable. The nonexchangeable space represents Ca^{2+} sequestered in subcellular storage granules, and alteration of Ca^{2+} binding and/or fluxes in the other compartments has an important role in the process of platelet aggregation and exocytosis (1). In subjects with Down's syndrome, blood platelets have decreased Na^+/K^+-ATPase activity and increased intracellular Na^+ (9). In erythrocytes, it has been reported that Na^+/K^+-ATPase is inhibited by increases in intracellular Ca^{2+} (2, 3), which may lead to raised intracellular Na^+ (13); thus, it seemed pertinent to determine the Ca^{2+} content of blood platelets from subjects with Down's syndrome as a possible factor contributing to decreased Na^+/K^+-ATPase activity.

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MATERIALS AND METHODS

Materials. $^{45}CaCl_2$ (0.6 mCi/mg) was obtained from New England Nuclear. $LaCl_2$ was spectrographic grade from Fisher

Chemicals. All other chemicals were from commercial sources and were the purest available.

Calcium determination. Blood samples were collected by a siliconized Vacutainer with EDTA anticoagulant. The erythrocytes were sedimented at 100 × *g* for 20 min, and PRP was transferred to a plastic tube and recentrifuged at 150 × *g* for 10 min to remove contaminating white cells. Platelets were separated from plasma at 100 × *g* for 20 min, and after decanting the plasma, the platelet pellet was gently resuspended in buffer (150 mM NaCl, 1.0 mM EDTA, 0.35% albumin, pH 6.9, with NaOH), and the platelets were harvested by centrifugation at 700 × *g* for 10 min. All centrifugations took place at room temperature. The final platelet pellet was resuspended in lanthanum buffer (150 mM NaCl, 20 mM Tris-maleate, 0.35% albumin, 0.2% $LaCl_3$, pH 6.4), transferred to an acid-washed glass beaker, and ashed at 450°C for 18 h. The ashed residue was dissolved in deionized water containing HCl, and Ca^{2+} concentration was determined by atomic absorption spectrophotometry. For each platelet sample, calcium standards and lanthanum blanks were run in parallel.

Calcium transport. Platelets separated from PRP by centrifugation were resuspended in imidazole-buffered saline (150 mM NaCl, 20 mM imidazole, 10 mM glucose, 2.5 mM $MgCl_2$, 0.05 mM ATP, 2.5 mM $CaCl_2$, 0.5% albumin) to a final platelet concentration of 2×10^{-9} platelets/ml. After 5-min preincubation at 36°C, 5 μCi $^{45}Ca^{2+}$ were added and the incubation continued for a further 90 min with gentle agitation. $^{45}Ca^{2+}$ uptake was stopped by adding 4 ml ice-cold imidazole buffer followed by centrifugation. The platelet pellet was washed twice with ice-cold buffer and dissolved in 1 ml of 1% Triton X-100. 0.5-ml aliquots were taken for scintillation counting. Platelets in PRP or buffers were measured on a Coulter counter. Down's subjects were young adults of both sexes living at home. Controls were laboratory staff matched for sex and age. Informed consent was obtained from individuals, parents, or guardians.

RESULTS

The data from individual subjects for total platelet Ca^{2+} (nanomoles $Ca^{2+}/10^{-9}$ platelets) are given in Table 1. The calcium content of normal platelets was found to be 174.2 ± 10.0 nmol/10^{-9} platelets, which is significantly higher than the value of 85.8 ± 5.9 nmol/10^{-9} platelets found in Down's syndrome platelets ($p < 0.0005$). Calcium uptake and/or exchange was also reduced in Down's syndrome. After 90-min incubation, the amount of $^{45}Ca^{2+}$ associated with the platelet, but resistant to washing with ice-cold buffer, was 1.17 ± 0.07 nmol/10^{-9} platelets for controls and 0.79 ± 0.06 nmol/10^{-9} for Down's ($t = 2.94$; $p < 0.005$) (Table 2).

DISCUSSION

The Ca^{2+} content of the human blood platelet exists in a number of intracellular "pools" with different rates of turnover (1), and although our values for Ca^{2+} concentration in normal platelets are similar to published values (16), the reduction found in Down's syndrome platelets could be due to metabolic disturbances in any, or all, of the Ca^{2+} pools.

Table 1. *Ca^{2+} content of platelets (nmol/10^{-9} platelets)**

Controls	Down's syndrome
Individual findings	
162.5, 202.5	85.5, 65.0
182.5, 117.5	92.5, 102.5
147.5, 277.5	95.0, 47.5
165.0, 167.5	135.0, 100.0
142.5, 192.5	102.5, 102.5
172.5, 172.5	75.0, 87.5
135.5, 220.0	87.5, 57.5
155.0	52.5
Average	
174.2 ± 10.01	85.83 ± 5.99
$p = 0.0005$	

* Paired findings in matched subjects.

Table 2. *$^{45}Ca^{2+}$ uptake by blood platelets following 90-min incubation (nmol/10^{-9} platelets)**

Controls	Down's syndrome
Individual findings	
0.90, 1.04	0.80, 0.65
1.07, 1.07	0.86, 0.68
1.50, 1.01	0.99
1.13, 1.65	
Average	
1.17 ± 0.07	0.79 ± 0.06
$t = 2.94$	$p = 0.001 < 0.005$

* Paired findings in matched subjects.

Calcium is loosely bound to the platelet surface where it is freely exchangeable with extracellular Ca^{2+} and removable by chelating agents (17). As there is little difference between $^{45}Ca^{2+}$ binding between normal or Down's platelets following short incubation times (data not given), it is unlikely that alterations in the surface binding of Ca^{2+} can explain the decreased levels in Down's syndrome. Approximately 80–90% of the total platelet Ca^{2+} is associated with the dense bodies (14), complexed with adenine nucleotides and serotonin (4, 18), and only slowly exchanges with extragranular Ca^{2+}. As subjects with Down's syndrome exhibit decreased transport and storage of serotonin (7, 8), a decrease in the number of serotonin storage granules could account for the low intracellular Ca^{2+}. While this report was in preparation, More *et al.* (10) reported that Down's platelets have a reduced number and volume of electron-dense bodies and that whole cell and dense body calcium levels are lower than normal, complementing our observations.

In addition to decreased Ca^{2+} content, our results also demonstrate decreased accumulation of $^{45}Ca^{2+}$ in Down's platelets, but whether this represents decreased Ca^{2+} transport operating at the level of the plasma membrane, or sequestering of Ca^{2+} by the dense tubular system (12), is at present under investigation. It is of interest that the Ca^{2+} system in the human platelet membrane appears analogous to that in synaptic membranes (5, 6) where the common requirements for Ca^{2+} are seen in receptor-binding transmitter release membrane pump for Ca^{2+}, and supports the use of the platelet for a peripheral model for the synaptosome (15). Further studies on Ca^{2+} metabolism may provide additional understanding of Down's syndrome at a molecular level.

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Application of Receiver-Operator Analysis to Diagnostic Tests of Iron Deficiency in Man

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Summary

The objective of the present report is to demonstrate the use of receiver-operator characteristics (ROC) analysis in the selection of diagnostic tests for iron deficiency in a specific population. Conventional ROC curves were prepared with true positive fraction (TPF) and false positive fraction (FPF) determined by the application of different cut-off points for four indicators of iron status. ROC plots were then transformed into normal deviate scales. The advantages of Gaussian transformation of TPF and FPF when underlying decision functions are normally distributed are: (i) the ROC curve is a straight line; and (ii) the separation between the two distributions and shape of these distributions can be simply quantitated as intercepts and slopes. In the present study, pretreatment hemoglobin concentration was the most robust diagnostic indicator of iron deficiency as operationally defined by a response of hemoglobin to iron treatment. Free erythrocyte protoporphyrin was a more sensitive and specific predictor than either serum ferritin or transferin saturation when a stringent operational definition of iron deficiency was used. These findings illustrate the utility of ROC analysis in discriminating between diagnostic indicators having different degrees of accuracy.

Abbreviations

FN, false negative
FP, false positive
Hb, hemoglobin
***N*, normal individuals**
***D*, diseased cases**
ROC, receiver-operator characteristics
TPF, true positive fraction
FPF, false positive fraction
FEP, free erythrocyte protoporphyrin
SF, serum ferritin
TS, transferrin saturation

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A wide range of laboratory tests is currently used to assess systemic iron status in man. However, normal biological variability, measurement error, and confounding factors such as intercurrent infection may adversely affect the diagnostic efficiency of these tests. Some of these problems are minimized when iron status is operationally defined by the degree of hematologic response to iron administration. In assessing an individual's iron status, a significant rise in circulating hemoglobin mass in response to iron treatment provides reliable evidence of antecedent iron deficiency. Hemoglobin response can also be used to monitor the diagnostic efficiency of other tests of systemic iron status.

The evaluation of a test's diagnostic efficiency requires assessment of its discriminative capacity in circumstances where the frequency and nature of its diagnostic errors can be unequivocally determined. A test's accuracy (ratio of correct decisions to total number of subjects tested) is of limited usefulness as a general index of diagnostic performance because it is strongly affected by disease prevalence (8).

If a test is to be used to discriminate iron-replete from iron-depleted subjects, some definitive diagnostic criterion is needed to allow evaluation of that test. In Figure 1, the performance of a hypothetical diagnostic test is examined. Diseased subjects, whose test result places them at the right of the cut-off point, *a*, will be FNs; normal individuals whose result is to the left of *a* will be FPs. The number of FPs can be reduced or eliminated by moving the cut-off *a* toward *b*, to the lower end of the distribution for *N*. However, as a result of eliminating FPs, the FN fraction will be increased. Likewise, the number of FNs can be eliminated by moving the cut-off *a* to *c*, the upper end of the distribution for *D*. The cut-off point can be positioned so as to maximize a test's diagnostic performance in a given clinical or epidemiologic context (8)