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Diagnosing Platelet Function Disorders by Electron Microscopy

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Hemostasis is defined as the arrest of bleeding or of circulation in a part, including the stagnation of blood. Factors essential for normal hemostasis include a number of proteins in the circulation called coagulation factors that may become activated via three different mechanisms (intrinsic, extrinsic, and common pathways), an intact vascular endothelium, and small anucleate blood cells called platelets (PLT). Conditions that affect hemostasis may be acquired or inherited and may result in abnormal thrombosis or hemorrhage, which are considered collectively as bleeding disorders. Bleeding disorders are reported to be rare with vonWillebrand Disease (vWD) considered the most common bleeding diathesis, affecting 1-2% of the general population. Acquired risk factors for abnormal clotting are extensive including obesity, smoking, vascular disease, trauma, and the use of a number of drugs to name just a few. Inherited thrombotic conditions are usually related to mutations or deficiencies of coagulation proteins in blood. Acquired bleeding tendencies are usually secondary to disease states such as liver or renal failure, bone marrow abnormalities, to dietary conditions leading to vitamin K deficiency, or to the use of a number of different drugs including aspirin. Inherited bleeding may be due to mutations or deletions of PLT receptors, deficiencies of PTL constituents, or defects inherent in the vasculature. To diagnosis a bleeding disorder, a patient's medical history is essential; for instance, abnormal bleeding generally has common symptoms including joint or deep tissue bleeding or mucocutaneous bleeding presenting as easy bruising, frequent nose bleeds, gum bleeding while brushing, and for women, heavy menstrual bleeding.

This presentation will address the use of microscopy to diagnosis bleeding disorders related to PLT dysfunction. To diagnosis PLT dysfunction, clinical symptoms will have been prominent and laboratory tests will have ruled out a quantitative etiology (low PLT count). Qualitative tests such as PLT aggregation and secretion assays may/may not provide a diagnosis (I.E., abnormal PLT response to ristocetin and/or determination of vonWillebrand factor and multimers for diagnosis of vWD). Our clinical lab receives blood samples to diagnosis patients having macrothrombocytopenia (giant PLTs and low PLT count) and patients suspected of having storage pool deficiency (SPD, absence or low numbers of PLT alpha or delta granules).

Platelet storage pool deficiency is thought to be a very rare bleeding diathesis. Alpha granule deficiency may be suspected when evaluating blood smears with a light microscope and identifying platelets larger than red blood cells that appear to be gray in color (Gray PLT Syndrome). Alpha granules usually number 50-80 in normal platelets and the diagnosis can be confirmed using electron microscopy when observing a diminished number of these granules. A delta (δ or dense) granule deficiency (δ-SPD) may be suspected when mucocutaneous bleeding symptoms cannot be explained by routine clinical lab tests. Platelet aggregation/secretion assays may demonstrate abnormal responses to PLT agonists including ADP and epinephrine but these assays may be unreliable, especially if a patient has ingested aspirin within two weeks of the test. The most well recognized δ-SPDs include Hermansky-Pudlak Syndrome (absence of δ granules) and Chediak Higashi Syndrome (lysosomal defect leading to recurrent infections and low δ granules); patients will have varying degrees of albinism for both syndromes. We have found a significant correlation of δ-SPD with patients diagnosed with Postural Orthostatic Tachycardia

Syndrome and we have data suggesting a significant etiology for menorrhagia also includes δ-SPD. A δ-SPD is readily diagnosed using electron microscopy to observe whole, air-dried platelets; fixation, processing, embedment and sectioning are not required. The technique and examples of a variety of platelet dysfunction disorders, as well as results of some of our research projects, will be discussed during the presentation.

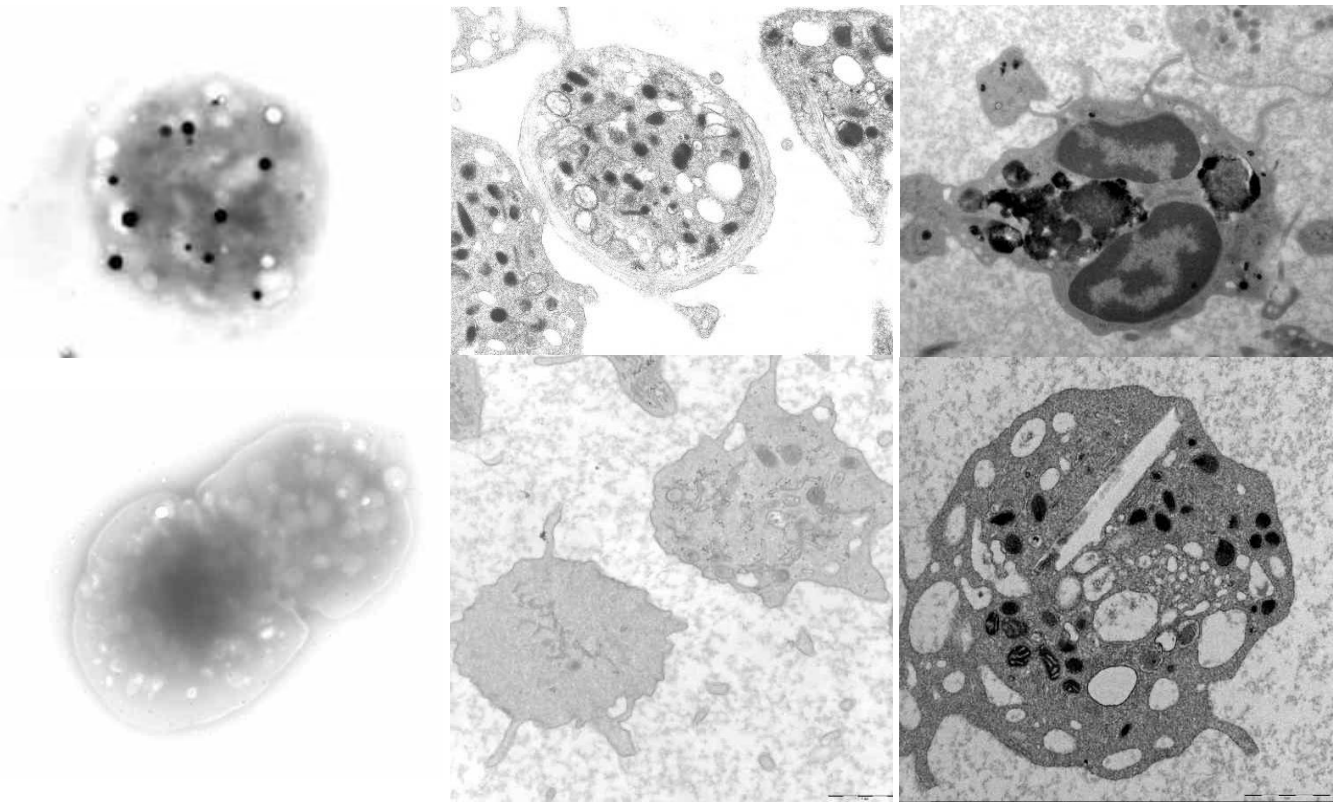

Figure 1. Whole mounted platelet demonstrating numerous dense (delta) granules (arrows).

Figure 2. Image of a whole mounted platelet devoid of delta granules characteristic of Hermansky-Pudlak Syndrome.

Figure 3. Normal platelet thin-sectioned demonstrates numerous alpha granules (arrows).

Figure 4. Platelets devoid of alpha granules characteristic of Gray Platelet Syndrome.

Figure 5. Neutrophil with abnormal, giant lysosomes occupying most of the cytoplasm. This is seen in Chediak-Higashi Syndrome which also presents as a bleeding diathesis with a deficiency of delta granules.

Figure 6. This image represents a very rare presentation of macrothrombocytopenia with normal dense granules but having diminished numbers of alpha granules and unusual rectangular cytoplasmic inclusions (arrow).

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