Megakaryoblastic Leukemia in a Dog

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Interpretation and Diagnosis

The blood picture from a routinely stained modified Giemsa blood smear with many blast cells allowed an immediate diagnosis of acute leukemia. The small pseudopods and occasional binucleated cells suggested this was likely megakaryoblastic leukemia (Fig 1-3). Finding mainly blast cells in a routine bone marrow aspirate smear and atypical megakaryocytes supported the diagnosis (Fig 4). Histologic sections of liver, lymph node, spleen and bone marrow illustrated a clear tendency of the blast cells to differentiate to megakaryocytes (Fig 5-8). Special staining was performed on bone marrow smears and histologic sections of liver. Deparaffinization, antigen retrieval, immunohistochemical staining and counterstaining was performed on the Bond maX™ Automated Staining System (Vision BioSystems™, Leica, Bannockburn, IL, USA) using the Bond™ Polymer Detection System (Vision BioSystems™, Leica, Bannockburn, IL, USA). Antigen retrieval was achieved using the Bond Epitope Retrieval Solution 1 (CD79a and CD61) and Solution 2 (Factor VIIIRA) for 20 min (both Vision BioSystems™, Leica, Bannockburn, IL, USA). The slides were incubated with a mouse monoclonal anti-CD79a antibody at a dilution of 1:100, a mouse monoclonal anti-CD61 antibody at a dilution of 1:50 and a rabbit polyclonal anti-factor VIIIRA antibody (all Dako, Carpinteria, CA) at a dilution of 1:100. The immunoreaction was visualized with 3,3-diaminobenzidine substrate (Vision BioSystemsTM) and sections were counterstained with haematoxylin. For negative controls, the primary antibodies were replaced with homologous non-immune sera. Only the CD61 stain gave very distinct staining (Fig 9-11). Factor VIII related antigen was weakly positive on bone marrow smears (Fig 12) and histology samples. CD79a appeared negative on a bone marrow stained smear.

Discussion

The original leukemia classification from routine blood smear was megakaryoblastic leukemia based on the formation of pseudopods (small hair-like to more blebbing type) and a tendency toward multinucleation or multilobed cells (Olsen 2008). The routine Giemsa bone marrow morphology had more of the multinucleated cells resembling megakaryocytes to support the diagnosis. Histologic sections of hematopoietic organs like bone marrow, liver, lymph node and spleen had a variable to large number of megakaryocytes to show differentiation in this direction. Thus the diagnosis of acute megakaryoblastic leukemia was reasonable certain with routine cytologic and histologic staining.

Of the special stains, the CD61 was most clearly positive both on the bone marrow aspirate and the liver histologic sections. CD61 is positive in acute myeloid leukemia megakaryocytic (AML-Meg) (Olsen 2008), as has been described previously in dogs (Colbatzky 1993, Ledieu 2005, Park 2006, Pucheu-Haston 1995). CD61 is negative in AML-E (erythroid), AML monocytic, AML MM (myelomonocytic), AML myeloid, AML-MD (minimally differentiated), B-ALL (B cell acute lymphoblastic leukemia) and T-ALL (Olson 2008). CD79a is part of the B-cell receptor complex and mainly restricted to B-ALL. CD79a was negative on bone marrow aspirate slides and hepatic sections, but neoplastic megakaryocytes in another case were reported to be positive for CD79a (Park 2006). Factor VIII related antigen was weakly positive in this case and in Ledieu's case, whereas another case had intense staining with factor VIII related antigen (Messick 1990). CD34 and CD41 were used to identify acute megakaryoblastic leukemia in another dog (Suter 2007). CD41 is glycoprotein GPIIb, specific for megakaryocytic linage, as is CD61. CD34 indicates a primary immature cell or acute leukemia.

The case presentation also allowed participants who received a DVD at the meeting to use the Aperio digital microscope to view digitalized histologic sections of the liver, lymph node, and bone marrow from the case.

References

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Suter SE, Vernau W, Fry MM, London CA. CD34+, CD41+ acute megakaryoblastic leukemia in a dog. Vet Clin Path 2007, 36:288-292.

Figures

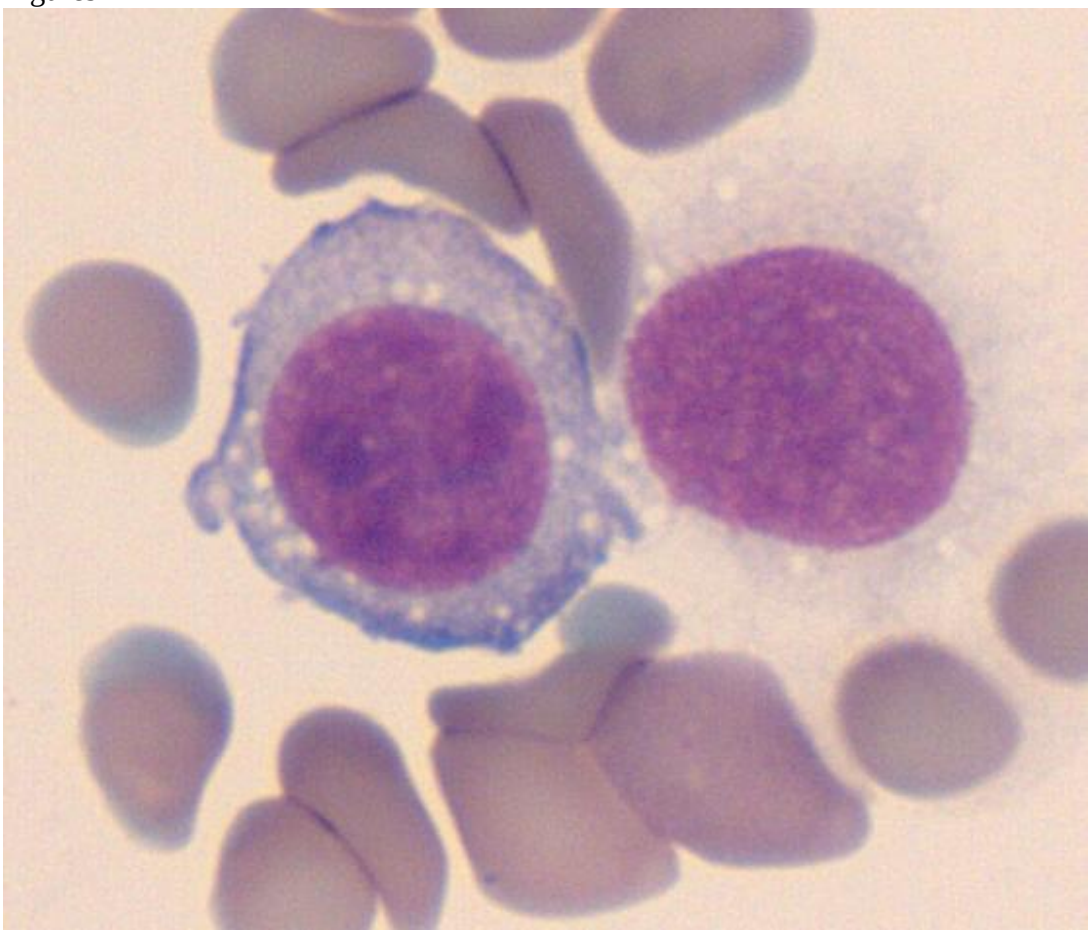

Figure 1 Peripheral blood smear with a large blast cell and a lysed cell. The blast cell has moderate to abundant cytoplasm, small indistinct red granules, several small vacuoles and tiny to moderate sized pseudopods. The nucleus is about 1.5 times larger than an erythrocyte, has fine chromatin and 2 or more nucleoli.

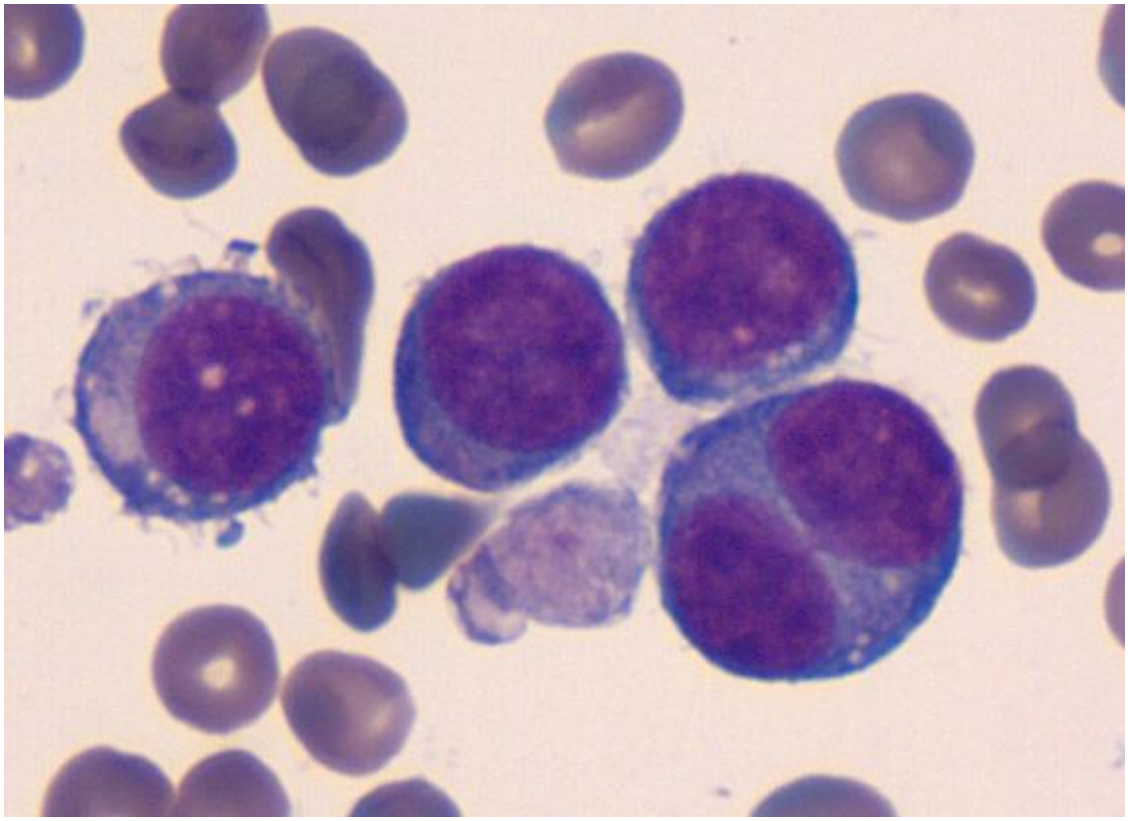

Figure 2 Peripheral blood smear with 4 large blast cells and a large cytoplasmic fragment. The blast cells have moderate to abundant cytoplasm, several small vacuoles and tiny hair-like or short blebbing type pseudopods. One cell is binucleated or bi-lobed with a filament between lobes.

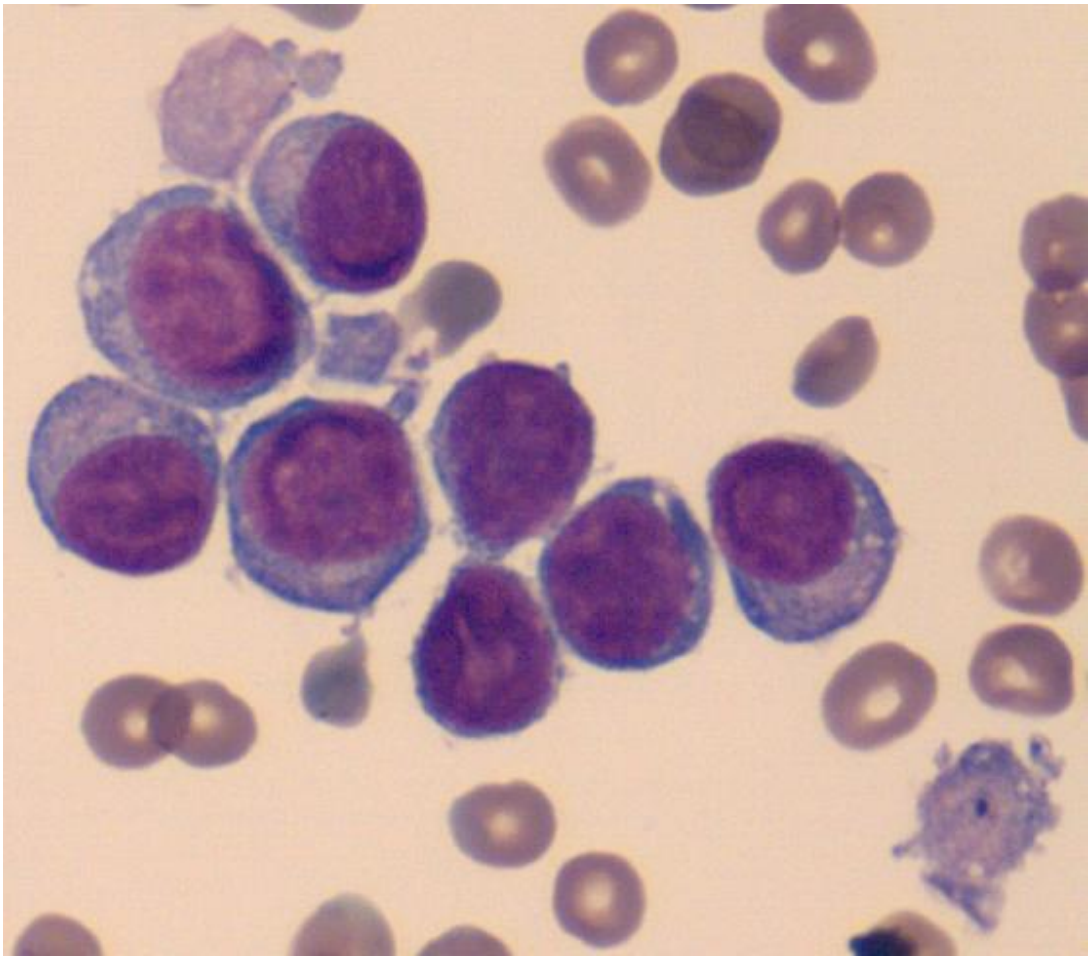

Figure 3 Peripheral blood smear with 8 blast cells and 2 large cytoplasmic fragments (giant platelets). The blast cells have moderate to minimal cytoplasm, small indistinct red granules, small vacuoles and tiny to moderate sized pseudopods.

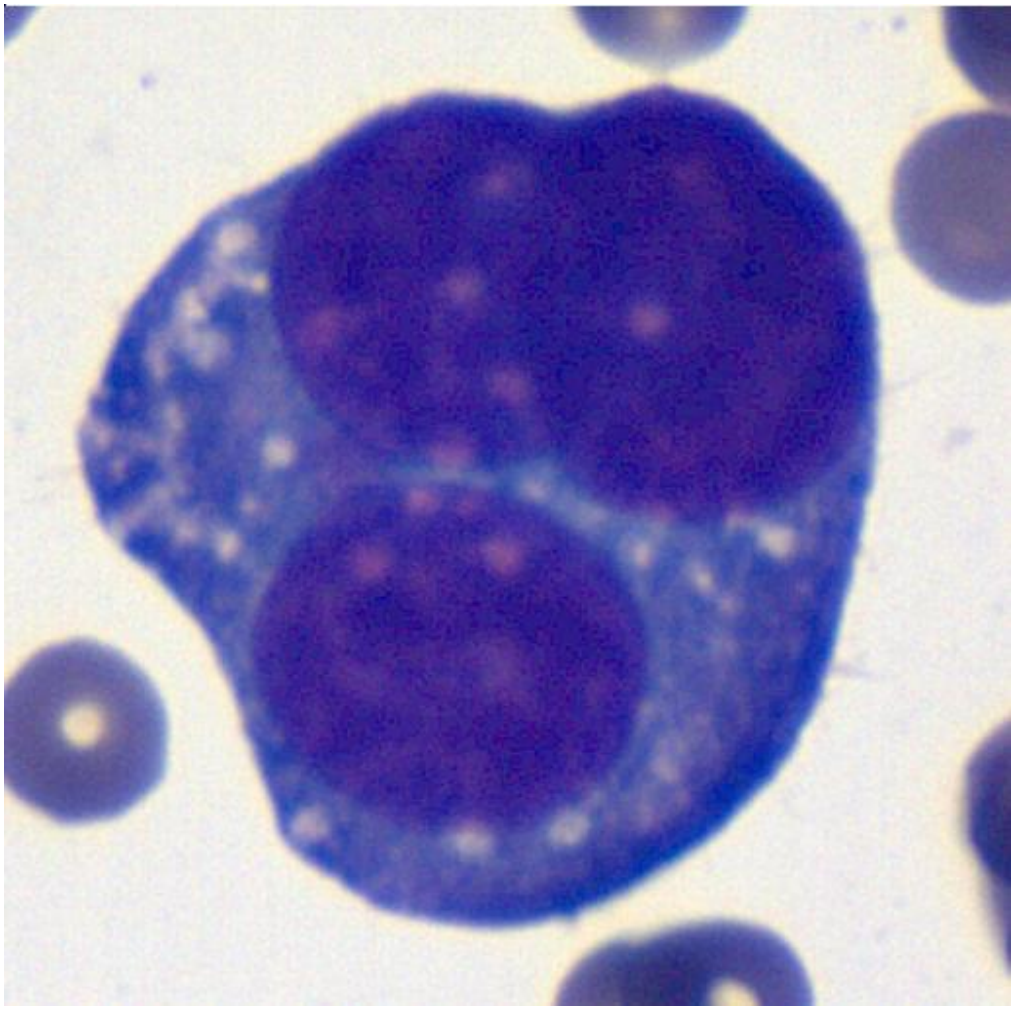

Figure 4 Bone marrow smear with an atypical megakaryocycte with 3 nuclei, small indistinct red granules, small vacuoles and thin hair-like pseudopods.

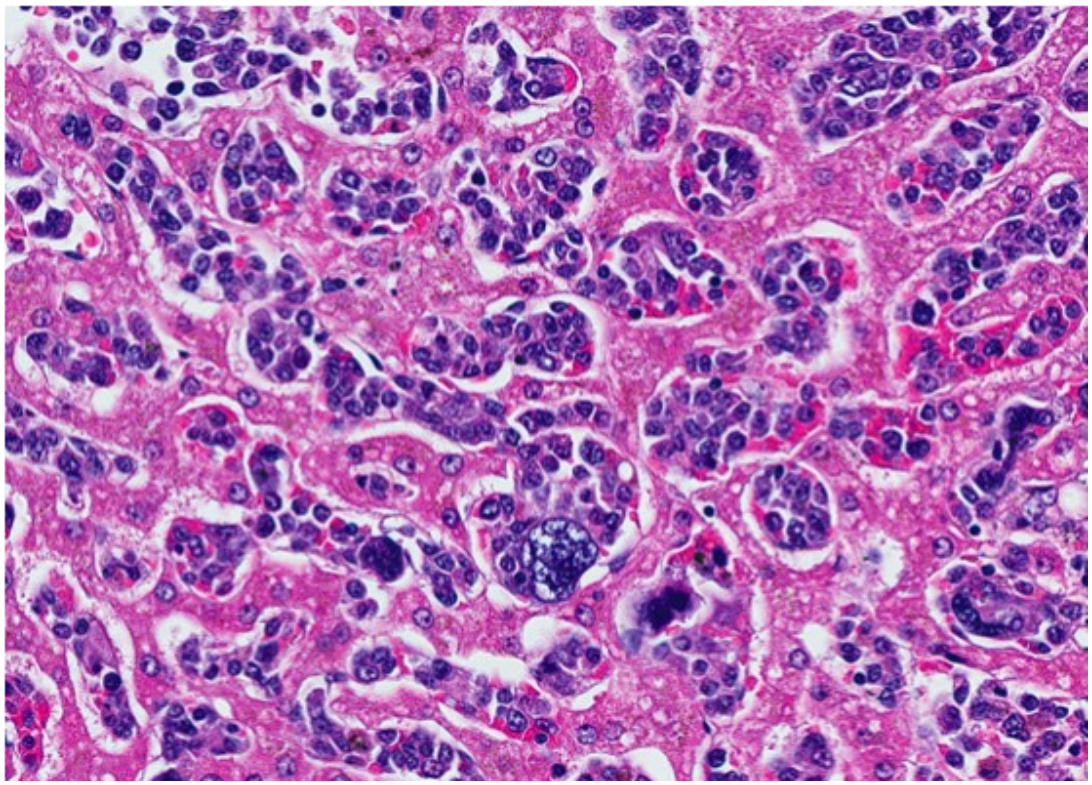

Figure 5 Histologic section of liver stained with H+E from the Aperio digitalized histologic section. The hepatic sinusoids are formed between pink hepatocytes with abundant cytoplasm which form linear columns or cords. The sinusoids are filled with darker smaller cells which are the leukemic cells. One of the neoplastic cells in the lower central area is a multinucleated megakaryocyte. Most of the invasive cells are blast cells with a single nucleus.

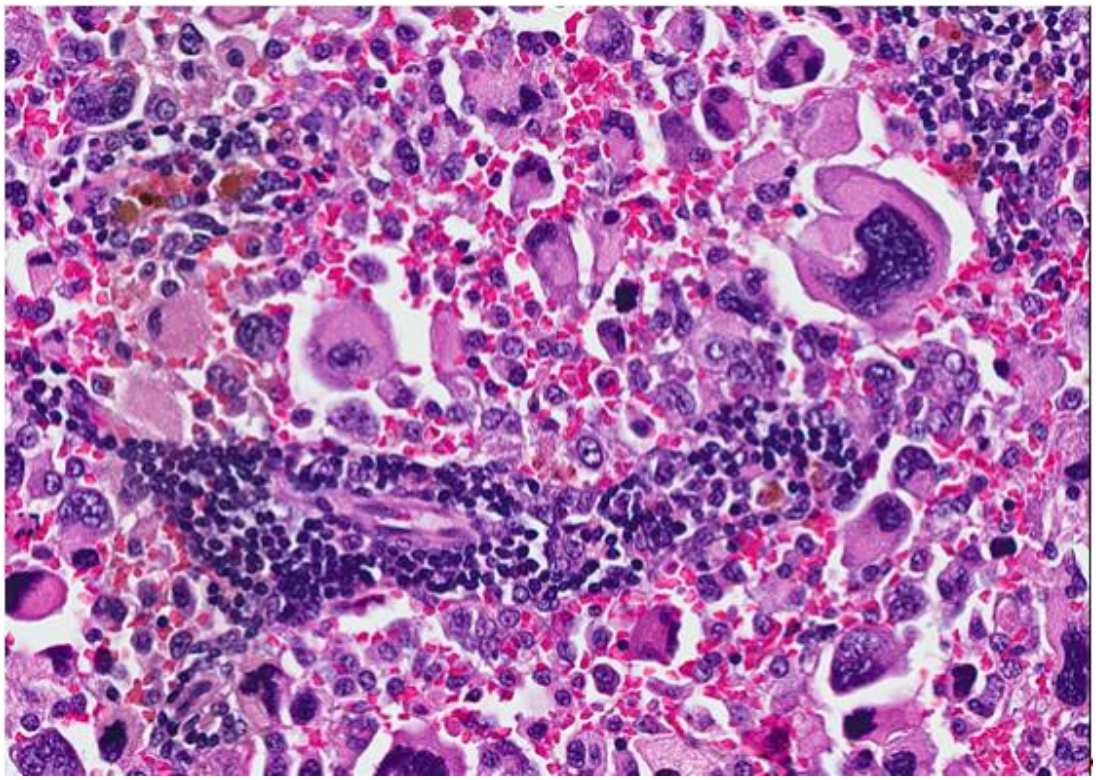

Figure 6 Histologic section of lymph node with extensive invasion of megakaryocytes and blasts in the medullary sinus from the Aperio digitalized histologic section. A medullary cord with normal lymphocytes around a blood vessel is in the middle. The lymph node section had the most differentiation to mature megakaryocytes.

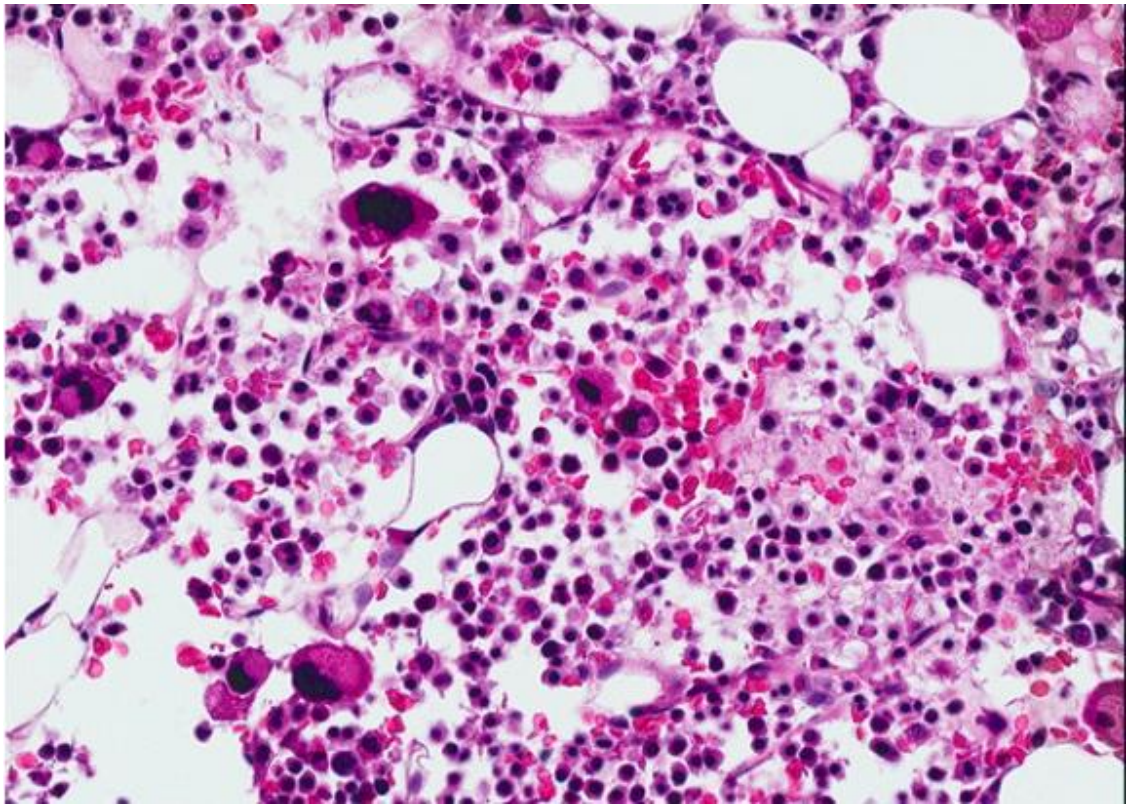

Figure 7 Histologic section of bone marrow. Most cells are blast cells with a single nucleus. There are a few megakaryocytes.

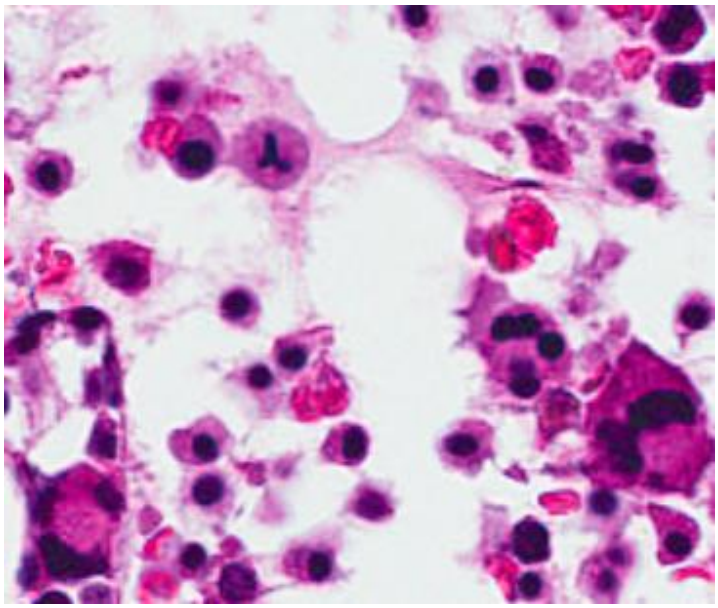

Figure 8 Histologic section of bone marrow. Most cells are blast cells with a single nucleus. There are 3 multinucleated megakaryocytes. Note a tripolar atypical mitotic figure in the upper left center.

Figure 9

Histologic section of liver stained with CD61 (platelet glycoprotein IIIa) positive cells. Most of the megakaryocyte type cells were positive (brownish-red) and about 10 % of the smaller blast cells. Photo by Dr. Matti Kiupel.

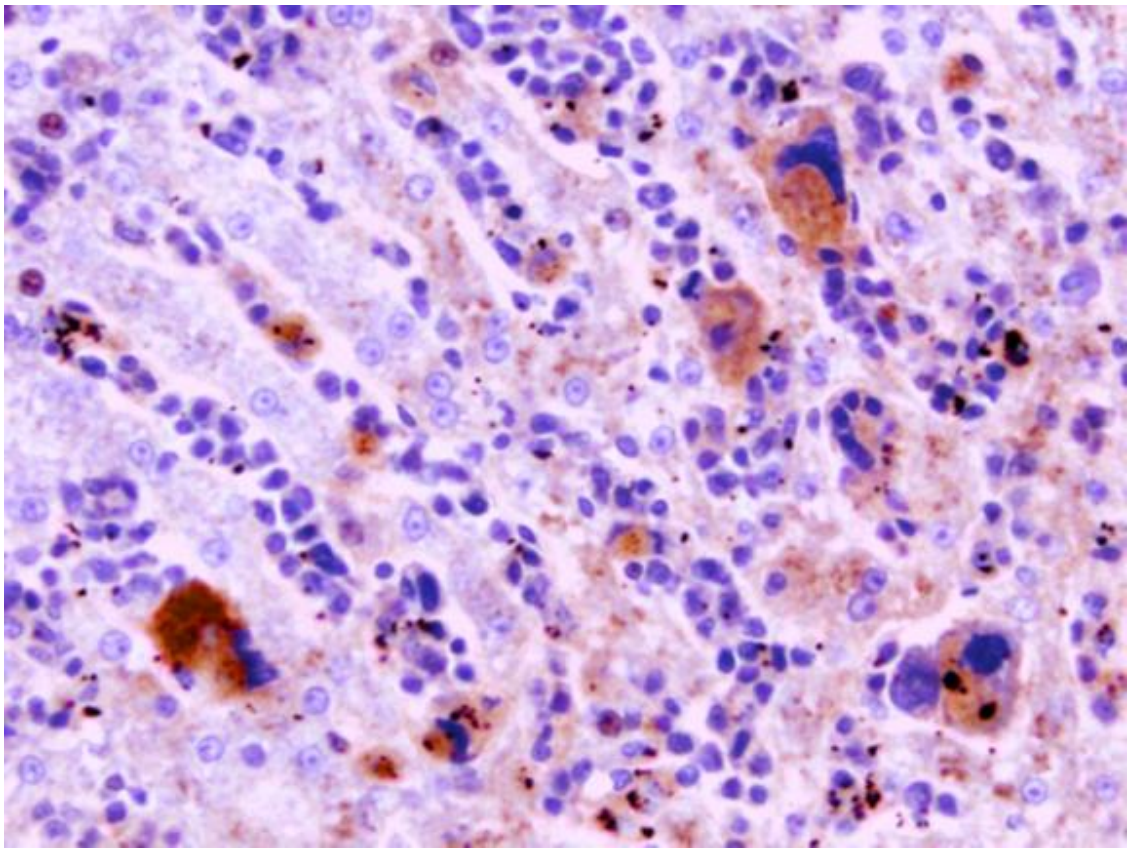

Figure 10

Histologic section of liver stained with CD61 (platelet glycoprotein IIIa) positive cells. Most of the megakaryocyte type cells were positive (brownish-red) and about 10 % of the smaller blast cells. Photo by Dr. Matti Kiupel.

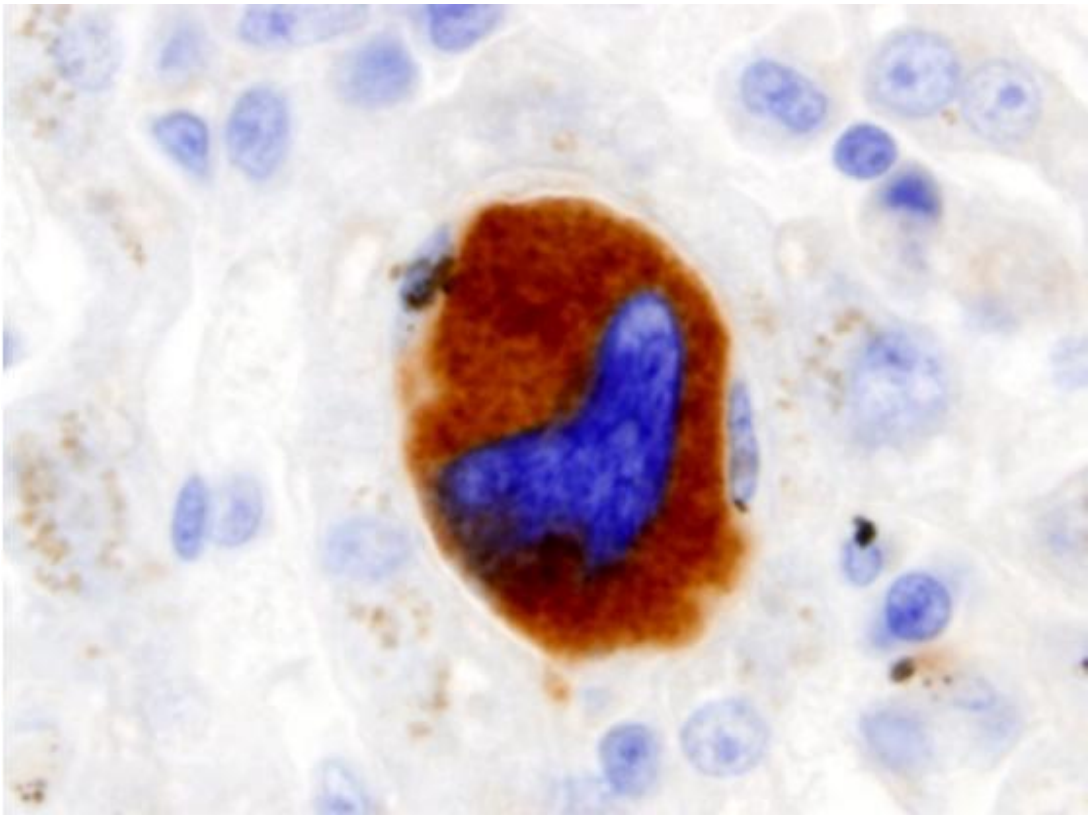

Figure 11

Smear of bone marrow aspirate stained with CD61 (platelet glycoprotein IIIa) has at least 6 positive cells (brownish-red) of the blast cells. A neutrophil at upper left was not positive.

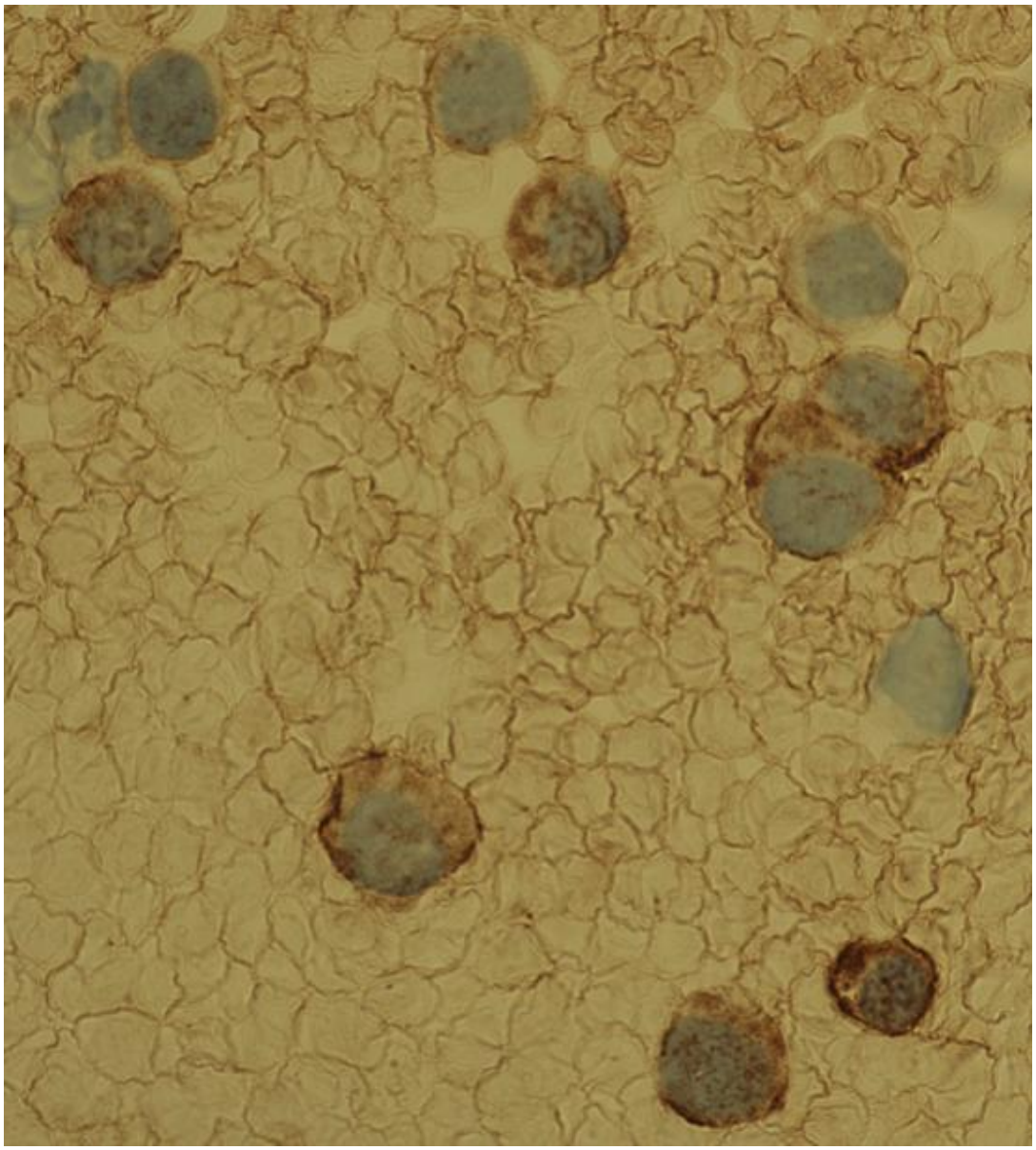

Figure 12

Smear of bone marrow aspirate stained with factor VIII related antigen which had some staining of the blast cells but not as distinctly as CD61 staining.