

Rouleaux Formation of RBC

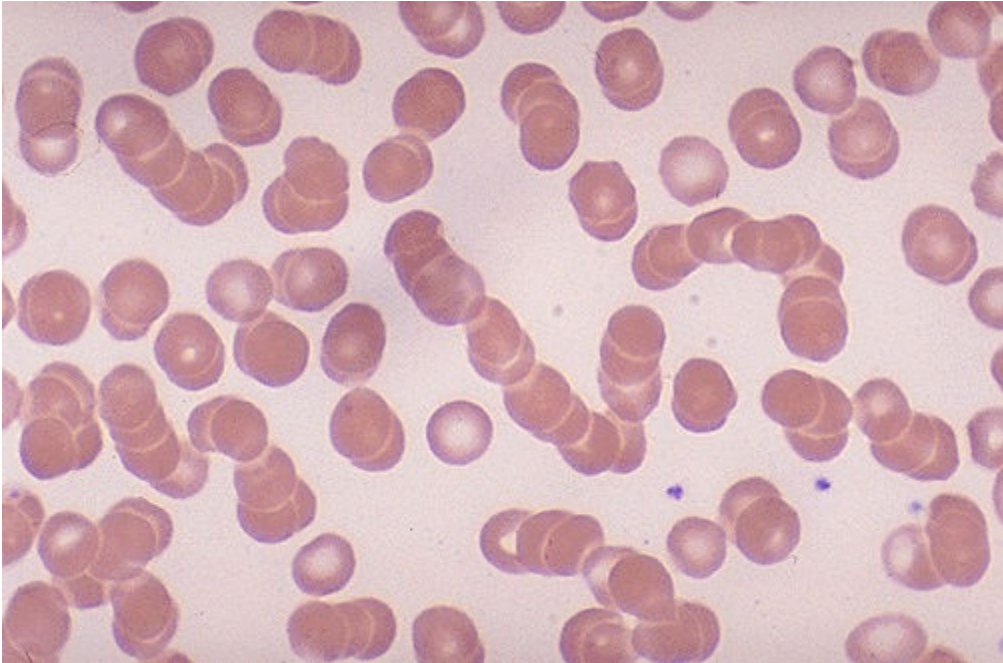
Rouleaux (singular is rouleau) are stacks of red blood cells (RBCs) which form because of the unique discoid shape of the cells in vertebrate body. The flat surface of the discoid RBCs give them a large surface area to make contact and stick to each other; thus, forming a rouleau. They occur when the plasma protein concentration is high, and because of them the ESR (erythrocyte sedimentation rate) is also increased. This is a non-specific indicator of the presence of disease.

Conditions which cause rouleaux formation include infections, inflammatory and connective tissue disorders, and cancers. It also occurs in diabetes mellitus and is one of the causative factors for microvascular occlusion in diabetic retinopathy.

The presence of acute phase proteins, particularly fibrinogen, interacts with sialic acid on the surface of RBC and allows the formation of rouleau. Anaemia, by altering the ratio of RBC to plasma, increases rouleaux formation and accelerates sedimentation. Rouleaux formation is retarded by albumin proteins.

Rouleaux formations are also adopted by spermatozoa as a means of cooperation between genetically similar gametocytes so as to improve reproductive success through enhanced motility and, therefore, fertilization capacity, e.g. in the guinea pig.

According to Smoluchowski, the kinetics of aggregation of colloids is based on the assumption that each particle is surrounded by a 'sphere influence'. Single spherical particles, which undergo Brownian motion collide and lead to sticking of particles. As aggregation proceeds, the average diffusion constant of the aggregate population decreases. The aggregation of red blood cells progresses in the same manner except that cells are biconcave rather than spherical.



The RBC's here have stacked together in long chains. This is known as 'rouleaux formation' and it happens with increased serum proteins, particularly fibrinogen and globulins. Such long chains of RBC's sediment more readily. This is the mechanism for the sedimentation rate, which increases non-specifically with inflammation and increased 'acute phase' serum proteins.

Rouleaux formation is the arrangement of red blood cells in fluid blood (or in diluted suspensions) with their biconcave surfaces in apposition, thereby forming groups that resemble stacks of coins. SYN: pseudoagglutination.

Causes of a high ESR include:

- malignancy:
 - malignant lymphoma
 - carcinomas of colon and breast
- haematologic:
 - multiple myeloma - a high ESR plus osteoporosis equals multiple myeloma until proved otherwise

- anaemia of acute or chronic disease, alone or combined with iron deficiency anaemia - not Fe deficiency alone.
- Macrocytosis elevates ESR.
- connective tissue disorders - especially:
 - systemic lupus erythematosus [normal in 5% of patients
 - rheumatoid arthritis
 - polymyalgia rheumatica
 - temporal arteritis
 - systemic sclerosis
- infections:
 - tuberculosis
 - acute hepatitis
 - bacterial
- others:
 - sarcoidosis
 - renal diseases - especially with azotemia
 - drug fever
 - hepatic cirrhosis
 - physiological increases in fibrinogen e.g. during pregnancy, also raise ESR levels.

The investigation of a patient with a high ESR and no clear explanation should include the following:

- full blood count
- immunoglobulin electrophoresis
- urea and electrolytes
- creatinine
- radiography of the chest and abdomen
- biopsy (e.g. bone marrow, temporal artery) may be indicated

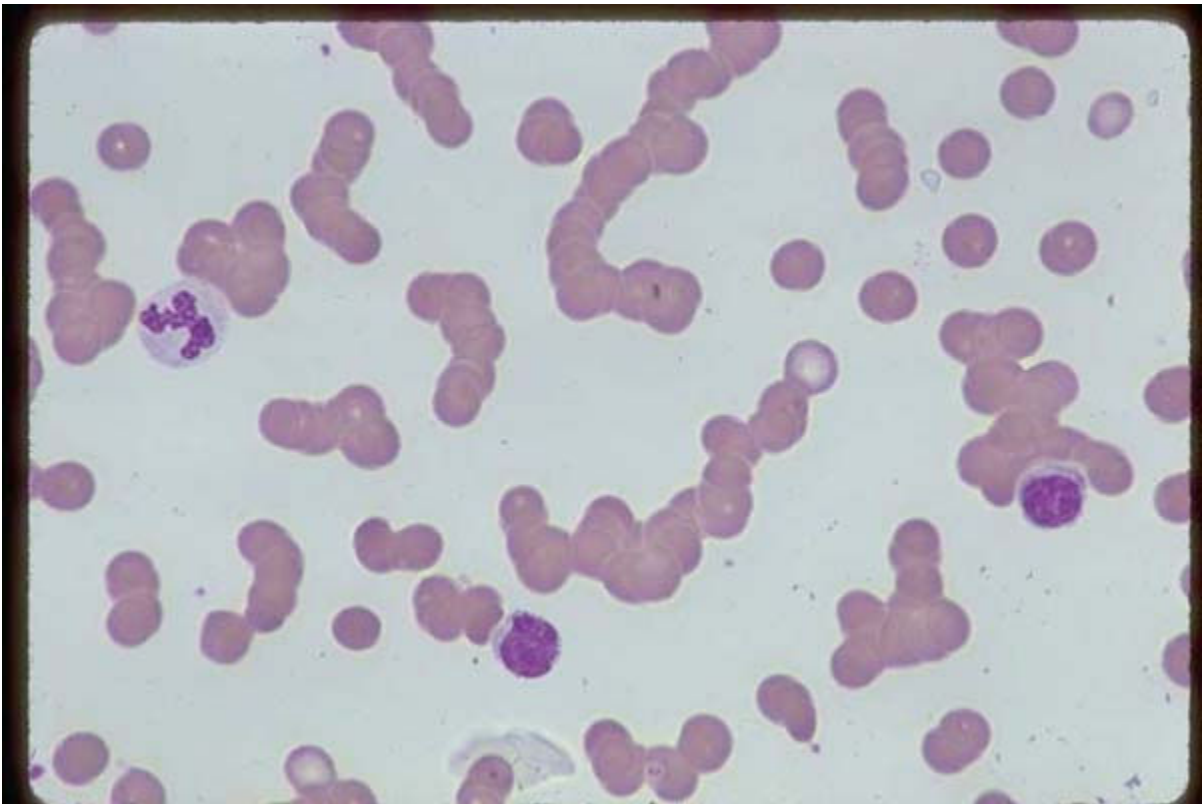
A multiple myeloma is a malignant neoplasm of plasma cells that arises in the bone marrow. Presentation is with anaemia, bone pain, skeletal destruction, pathologic fractures, or Bence Jones proteinuria.

In the majority of cases, complete immunoglobulins are secreted together with an excess of Ig fragments. In up to one quarter of cases, Ig fragments only occur and the condition is referred to as light chain disease or Bence Jones myeloma.

Multiple myeloma

Much rouleaux formation of the red cells is in this thick area of red cell distribution. This is pathologic rouleaux in contrast to normal stacking of red cells in a thick area of a normal blood smear. The background has a bluish tinge. Two small lymphocytes and one mature neutrophil are in the field.

Multiple myeloma (Blood - 50X)



The stacking of cells (rouleaux formation) facilitates the rate of red cell sedimentation, a phenomenon that may be seen on a peripheral smear. (*The appearance of rouleaux may be artificially caused by a poor preparation of the smear or by viewing the slide in a thickened area.*) When

rouleaux formation is truly present, it is caused by an increase in cathodal proteins, such as immunoglobulins and fibrinogen. Although myeloma and macroglobulinemias are first considered by hematologists, other causes occur more frequently, such as acute and chronic infections, connective tissue diseases, and chronic liver disease.

Red blood cells aggregate face-to-face to form long, cylindrical, straight chains and sometimes branched structures called rouleaux. When rouleaux grow large, they may contain rings or loops and take on the appearance of a network. Fibrinogen is secreted by liver and is present in blood.

The reason rouleaux do not form in vivo is that they only form under low shear stress conditions. With blood flow, the cross-sectional fluid velocity gradient in the blood vessel results in shear stress levels that exceed the threshold for rouleaux formation.

Case History

Tilak Ram, a male aged 45 years, was admitted to this hospital in October, 1968, with gradually increasing swelling of the right orbit for 8 months prior to admission. Along with the appearance of the right orbital swelling, the patient experienced generalised weakness and pains in the whole of his body. Two months before admission he started feeling moderate intensity of pain in the left orbit also. The patient developed symptoms of acute confusional state during his stay in the hospital. He had small-pox infection at the age of 10 years when he lost his left eye.

General physical examination showed anaemia and marked emaciation of the whole body with generalised tenderness of the chest bones and the spine. Except in the right orbital region no other swelling was noticed anywhere else on the body. Small-pox scar marks were seen sparsely distributed all over. No abnormality was detected in the cardiovascular, respiratory and central nervous

systems and in the gastrointestinal tract. Pyorrhoea alveolaris with absence of teeth in the upper and lower jaws was present.

Examination of the eyes:

The right eye showed eccentric proptosis with forward, downward and inward displacement of the eyeball. The proptosis was non-compressible, non-pulsatile, with slight tenderness over its upper and outer limits. The colour of the skin over the lids and the neighbouring areas was normal. The proptosis was due to two almond shaped swellings situated in the superolateral and inferolateral orbital margins which were extending to the right temporal region. The skin over the swellings was mobile.

Mild pressure on the eyelids resulted in subluxation of the eye. Movements of the eyeball were restricted in upper, outer and lower directions. The conjunctiva showed moderate pale white chemosis. Slight xerosis of the lower 1/3 of the cornea was present. The pupil reacted sluggishly to light and accommodation. The examination of the fundus could not be done because of the marked haziness of the vitreous. The vision gradually deteriorated. The left eye showed enophthalmos due to phthisis bulbi resulting from small-pox involving the eye. The eyeball was completely shrunken and the cornea was totally leucomatous with calcification over its surface. There was no tension in the eye. On eversion of the upper lid a small nodular swelling was visible in the left fornix. It was difficult to reach its posterior limit. The swelling was separate from the eyeball. The movement of the eyeball was negligible. There was no obvious displacement of the eye. The vision was completely absent.

Radiological examination:

The right orbital cavity was enlarged and there was extensive bony erosion of, the superior and lateral orbital margins with slight rarefaction of the orbital plate of the frontal bone. A small translucent punched out area was seen in the region of the greater

wing of the sphenoid. The left orbit showed a large translucent area and rarefaction in the orbital plate of the frontal bone. Small bony erosion was observed near the superomedial margin of the orbital rim. The cavity was normal in size. In the skull, generalised rarefaction with numerous rounded discrete translucent areas of varying sizes was shown.

The examination of the spine, chest bones and the pelvis showed generalised rarefaction with characteristic multiple punched out translucencies. Few punched out osteolytic and rarefied areas were also seen in the long bones of the upper and the lower extremities. Pathological fractures were seen at the upper end of the left fibula and the 3rd lumbar vertebra.

Laboratory findings:

Hemoglobin was 8.00 gm, total leucocytes 10,000/emm with a differential count of 65% polymorphs, 25% lymphocytes, 7% monocytes and 3% eosinophils. Peripheral blood film showed increased rouleaux formation of red cells and a moderate degree of hypochromia. No immature cells were seen. Platelets were normal in number and morphology. Bleeding and clotting time were within normal limits. Urine examination showed albuminuria (3 plus) and several hyaline and granular casts. Bence Jones proteins were absent on repeated examinations. Total serum proteins were 7.5 gm% with albumin 3.4%, alpha₁ 0.35 gm%, alpha₂ 0.35 gm%, Beta 0.2 gm%, Gamma 3.2 gm%. No myeloma proteins were seen. Serum calcium was 11.2 mg.

Histopathological examination:

The biopsy material consisted of a few small soft tissue pieces. H. E. stained paraffin section showed sheets and groups of tumour cells ovoid to polygonal in shape with a moderate amount of cytoplasm, each having a single usually large and eccentric nucleus. Chromatin pattern in some of the cells showed a typical cartwheel arrangement.

Occasional cells also showed a para-nuclear vacuole or nucleolus. A rare mitotic figure of binucleated cell was also seen.

Sternal marrow examination:

Cellularity was moderately good with a M. E. ratio of 3:1. Numerous myeloma cells and mature plasma cells either in clumps or singular were seen. The reaction was normoblastic.

Treatment

Apart from supportive treatment in the form of repeated blood transfusions no other form of treatment was considered worthwhile in view of the extensive involvement of the skeletal system. Radiotherapy to the proptosed eye was also withheld considering the existing visual disability as a result of previous phthisis bulbi in the other eye. Operative interference was abandoned because of diffuse infiltration of myelomatous deposits in the orbits and deteriorating condition of the patient.

In view of the presence of eccentric proptosis with marked generalised cachexia and anaemia the possibilities of a malignancy with secondaries in the orbit and a primary growth of lacrimal gland were initially considered.

Multiple myelomatosis was not thought of since the involvement of orbit in this disease is extremely rare. However, since the patient complained of generalised bone pains and as no evidence of a growth elsewhere was found on physical examination, skiagrams of the skeletal system were taken to exclude this disease. The x-rays revealed typical bone involvement of myelomatosis and the diagnosis was ultimately confirmed by other investigations.

The patient had enophthalmos of the left eye which had resulted from phthisis bulbi due to previous smallpox infection. Since there was no obvious swelling in this eye the involvement of the left orbit

was missed initially. Moreover in none of the reported cases of myelomatosis, bilateral orbital involvement has been described.

In the present case, however, subsequent examination revealed a small nodular swelling in the upper fornix which was confirmed on radiological and histopathological examinations to be due to myelomatous deposit. This therefore, probably is the only reported case in recent ophthalmic literature showing a bilateral orbital involvement.