

* CHAPTER 22

Sideroblastic anaemias

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1. Introduction

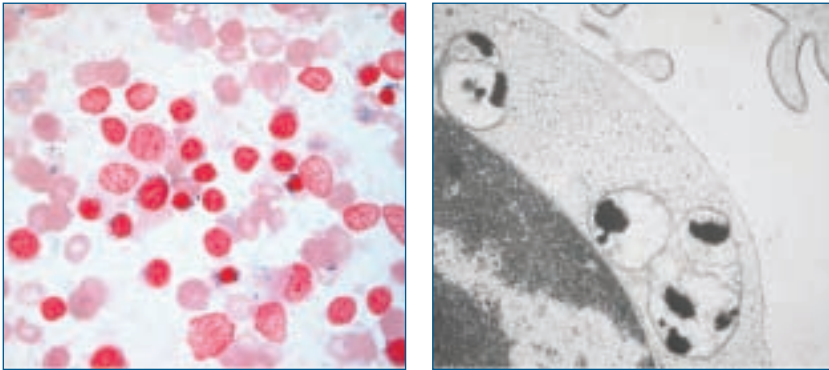
Sideroblastic anaemias (SA) are a heterogenous group of disorders characterised by the presence of ring sideroblasts in the bone marrow (BM). This cytomorphological feature is attributable to massive iron accumulation in the mitochondria of erythroblasts. Current classification schemes distinguish between *inherited/congenital* and *acquired* forms of SA.

The inherited/congenital forms often have a well-characterised mutation of nuclear or mitochondrial DNA affecting the haem synthetic pathway or other mitochondrial functions.

The acquired forms of SA are more frequent. They can either be *reversible* if caused by drugs or malnutrition, or can be part of a *clonal* BM disease called myelodysplastic syndrome (MDS). In the latter cases, the acquired mutations causing the sideroblastic phenotype are largely unknown. Treatment of acquired clonal SA is mainly supportive (blood transfusions, haematopoietic growth factors, iron chelation). Haematopoietic stem cell transplantation is rarely performed.

SA is defined as an inherited or acquired form of anaemia which on light microscopy (Prussian blue or Perls' staining) is characterised by the presence of ring sideroblasts, i.e. erythroblasts with a ring of iron granules around the nucleus. The iron granules usually cover more than a third of the nuclear perimeter. On electron microscopy, they correspond to iron-laden mitochondria. For a diagnosis of SA, ring sideroblasts must make up at least 15% of erythroblasts in the BM (Figure 1).

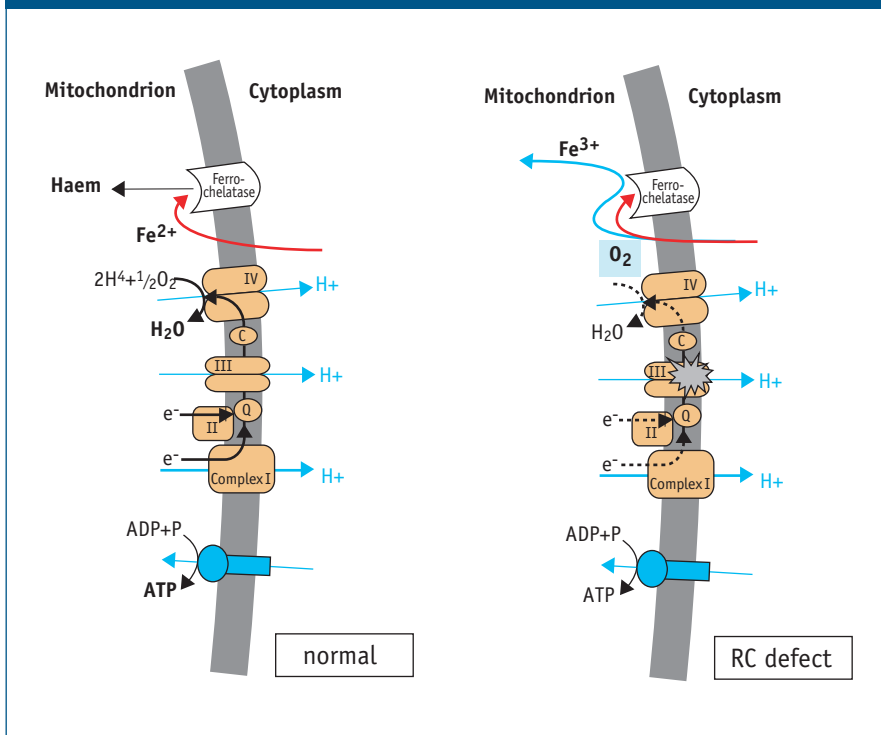
Figure 1: Light and electron microscopy of ring sideroblasts



Perls' staining of the bone marrow of a patient with refractory anaemia with ring sideroblasts (RARS) (left); Electron microscopy showing iron deposits in perinuclear mitochondria (right). From ref. (1).

In erythroblasts, iron is mainly used for haem synthesis. The haem biosynthetic pathway starts and ends in the mitochondria. The first step is carried out by the erythroid-specific enzyme delta-aminolaevulinic acid synthase 2 (ALAS2). This enzyme is under the control of two iron regulatory proteins (IRP1 and IRP2) acting on the ALAS2 iron responsive element (IRE), which is not present in non-erythroid ALAS1. The last step of haem synthesis is the insertion of Fe^{2+} into protoporphyrin IX, catalysed by ferrochelatase (Figure 2).

Figure 2: Working hypothesis on pathological iron handling in acquired idiopathic sideroblastic anaemia



During the final step of haem synthesis, ferrochelatase processes ferrous iron (Fe^{2+}) but cannot utilise ferric iron (Fe^{3+}) (2). In ring sideroblasts, mitochondrial iron accumulates as ferric iron (Fe^{3+}), mainly bound to mitochondrial ferritin (3, 4), not readily available for haem synthesis. This may be attributable to a failure of the respiratory chain (RC) to effectively remove oxygen from the mitochondrial matrix. A respiratory chain defect would decrease O_2 consumption and thereby increase O_2 concentration in the mitochondrial matrix. If iron, after crossing the inner mitochondrial membrane as Fe^{2+} (5-7), becomes oxidised ($\rightarrow\text{Fe}^{3+}$), it will be rejected by ferrochelatase and will thus accumulate in the mitochondrial matrix.

Iron is transported from the cytoplasm into the mitochondria by mitoferrin (MFRN). Zebrafish with a homozygous mutation of MFRN have severe hypochromic anaemia and no iron detectable in the mitochondria (8). Mitochondria have a specific type of ferritin, which has no IRE (9). This mt-ferritin is undetectable in normal erythroblasts but abundant in SA patients. It probably helps to protect mitochondria from oxidative damage.

Besides haem synthesis, mitochondria also harbour the iron-sulfur cluster assembly machinery. Fe/S cluster-containing proteins are found:

- in the mitochondria: components of the respiratory chain; ferrochelatase; enzymes of the citric acid cycle (aconitase, succinate dehydrogenase)
- in the cytoplasm: cytoplasmic aconitase/iron-regulatory protein 1
- in the nucleus: human endonuclease III homologue 1 (hNTH1).

Derangements of Fe-S cluster biosynthesis can cause human disease (10), and it is possible that they play an important role in the pathogenesis of SA.

2. Inherited sideroblastic anaemias

Inherited SAs can be classified as X-linked, autosomal, or attributable to mitochondrial DNA mutations (see Table 1).

X-linked	X-linked sideroblastic anaemia (XLSA) X-linked sideroblastic anaemia with ataxia (XLSA/A)
Autosomal	Glutaredoxin-5 deficiency Thiamine-responsive megaloblastic anaemia (TRMA) Associated with erythropoietic protoporphyria (EPP) Myopathy, lactic acidosis and sideroblastic anaemia (MLASA)
Mitochondrial DNA	Pearson syndrome

Adapted from (11) with modifications.

2.1 X-linked sideroblastic anaemias

2.1.1 XLSA

XLSA due to mutations in ALAS2 has served as a paradigm for all forms of SA. The first cases of XLSA were reported in 1945 by Cooley, in two brothers from a large family in which the inheritance of the disease was documented through six generations (12). In 1992, the first mutation in the ALAS2 gene was reported in a

male with pyridoxine-responsive anaemia, without a family history of anaemia (13). The disorder is the most common among the hereditary SAs. More than 20 different missense mutations (single base changes) of the ALAS2 gene have been described to date, which lead to decreased protoporphyrin and haem synthesis. The essential features of the disease include:

- hypochromic microcytic anaemia, often with Pappenheimer bodies (iron-positive erythrocytic inclusions) and two discrete populations of red blood cells, one microcytic and the other normocytic;
- an X-linked pattern of inheritance, with a predominance of males related through the maternal lineage;
- the presence of marrow ringed sideroblasts;
- clinical improvement in some cases with pyridoxine supplementation when the mutation disrupts the catalytic association between ALAS2 and pyridoxal phosphate;
- systemic iron overload, since ineffective marrow erythropoiesis secondary to decreased haem production induces increased iron absorption from the gastrointestinal tract (14, 15).

Erythrocyte Zn-protoporphyrin levels are normal. The age of clinical onset of the disorder can vary from *in utero* to the ninth decade. So-called "late onset" XLSA patients are often misdiagnosed as having the acquired form of SA. Two mechanisms appear to be relevant for the late manifestation of XLSA. The first is an age-dependent decline in pyridoxine bioavailability in elderly individuals, unmasking a latent pyridoxine-responsive defect in ALAS2. The second mechanism is unique to females with the disease and involves preferential expression of the mutant compared to the wild-type allele. Normally, the process of X-chromosome inactivation occurs as a stochastic event and leads to the expression of mutated and wild-type alleles in roughly similar cell numbers. Preferential or skewed expression ("Lyonisation") of the mutant allele might evolve as a stochastic event during ageing or could be inherited as a Mendelian trait, known as familial skewed X-inactivation (16).

The following is a typical case of ALAS2 mutation leading to SA: a 40 year-old man with microcytic anaemia, (Hb 7.9 g/dL; MCV 58fL), high serum ferritin and transferrin saturation 88%. Peripheral blood examination revealed a dimorphic erythroid population with basophilic stippling. BM aspiration showed numerous ring sideroblasts. Treatment with vitamin B6 (pyridoxine) 200 mg/d raised Hb to 12.5 g/dL in 10 weeks. Sequencing of the ALAS2 gene revealed the Ile289Thr mutation (17).

2.1.2 XLSA with ataxia (XLSA/A)

In 1985, Pagon et al. described a rare form of SA associated with neurological symptoms (18). To date, four families have been reported with this disorder.

Genetic studies demonstrated linkage to Xq13 and subsequent mutation analysis has demonstrated missense mutations in ABCB7 (19). In one patient with this disorder, ataxia was present from birth. This was associated with microcytic anaemia (Hb 10.8 g/dL; MCV 62.2 fL) with low serum iron, normal transferrin saturation and increased sTfR and erythrocyte protoporphyrin levels. A moderate iron overload developed (ferritin 622 µg/L) by the age of 30 years. Brain MRI showed cerebellar hypoplasia. BM aspiration and Perls' staining revealed numerous ring sideroblasts. Sequencing of the ABCB7 gene showed a missense mutation in exon 10. ABCB7 is a mitochondrial protein involved in the transport of a component required for the maturation of Fe-S cluster proteins from mitochondria to the cytoplasm (20, 21). The exact role of the ABCB7 protein in SA pathogenesis is not known, but it is possible that failure to export the Fe-S clusters might lead to accumulation of iron from excess intramitochondrial Fe-S clusters that are readily degraded.

2.2 Autosomal sideroblastic anaemias

2.2.1 Glutaredoxin-5 deficiency

Recently, the case of a 44 year-old man with severe microcytic anaemia (Hb 8.9 g/dL; MCV 59 fL) was described. The patient had iron overload (TF saturation 52%; ferritin 1100 µg/L). BM examination revealed erythroid hyperplasia with 28% ringed sideroblasts. The patient had a homozygous mutation in the glutaredoxin 5 gene which interferes with intron 1 splicing and drastically reduces glutaredoxin (GLRX5) RNA. GLRX5 has an essential role in the synthesis of Fe-S clusters. It is believed that by insufficient biogenesis of mitochondrial Fe-S clusters, with a subsequent increase in the IRE-binding form of IRP1, ALAS2 synthesis is suppressed resulting in impaired haem synthesis and mitochondrial iron accumulation (22).

2.2.2 Thiamine-responsive megaloblastic anaemia (TRMA)

TRMA is a rare autosomal recessive disorder defined by the triad of megaloblastic anaemia with ringed sideroblasts, non-type 1 diabetes mellitus, and progressive sensorineural deafness (23). In 1999, three groups identified the responsible gene on chromosome 1 (1q23.3), namely SLC19A2 (24-26). This gene codes for a transmembrane thiamine transporter (SLC19A2). Thiamine is necessary for four enzymes in mammalian cells: pyruvate dehydrogenase and α -ketoglutarate dehydrogenase of the Krebs cycle, transketolase, and branched chain keto-acid dehydrogenase. Since α -ketoglutarate dehydrogenase provides succinyl CoA, one of the two substrates for ALAS, decreased thiamine levels could lead to decreased haem biosynthesis. In addition, a defect in the transketolase pathway, which is necessary

for *de novo* ribose and consequently nucleotide synthesis, might account for the megaloblastic erythropoiesis (27). Inderneel et al. recently obtained further evidence for defective deoxyribose and haem synthesis from the study of these pathways in mice defective for the orthologous *slc19a2* gene using targeted gene disruption (28).

2.2.3 Erythropoietic protoporphyria (EPP)

This autosomally inherited disease is due to mutations in the ferrochelatase gene and leads to skin photosensitivity and liver disease (see Chapter 27). Anaemia is generally mild and BM aspirations are not routinely performed in these patients. In ten patients marrow ring sideroblasts with typical mitochondrial iron deposits were observed (29, 30).

2.2.4 Myopathy, lactic acidosis and sideroblastic anaemia (MLASA)

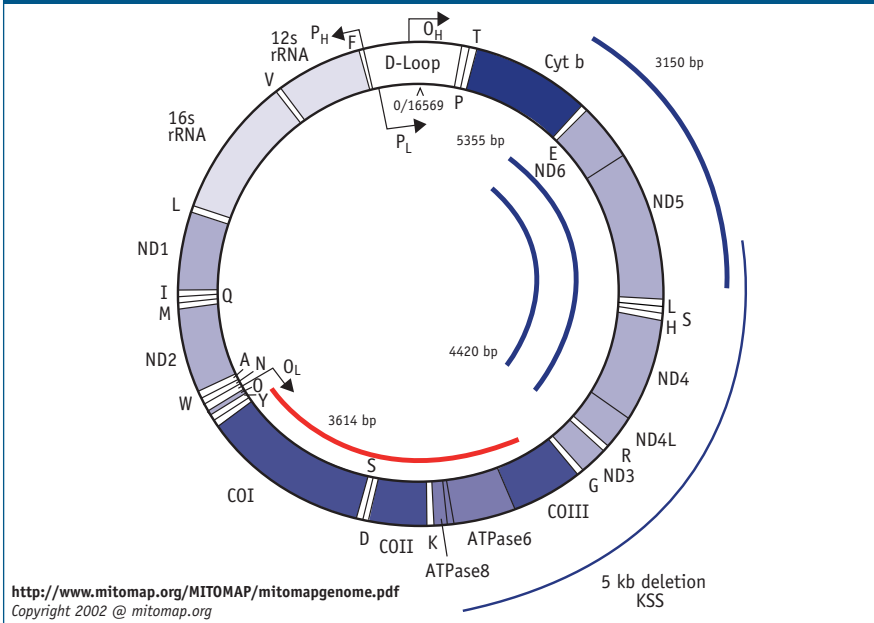
MLASA is a rare autosomal recessive disorder of oxidative phosphorylation and iron metabolism. Patients with MLASA present with weakness and anaemia in late childhood and may become transfusion-dependent. Positional cloning studies located the responsible gene to chromosome 12 (12q24.33) in a region containing 21 different genes (31). Recently, a homozygous missense mutation in the pseudouridine synthase 1 gene (*PUS1*) was found in all patients and deficient pseudouridylation of mitochondrial tRNAs was proposed as the pathophysiological mechanism leading to SA (32).

2.3 SA attributable to mutations in mitochondrial DNA

2.3.1 Pearson syndrome

Pearson syndrome is characterised by severe, transfusion-dependent SA in childhood, exocrine pancreatic insufficiency, and persistent metabolic acidosis with high blood lactate (33). Most patients do not survive beyond the age of three years. In survivors, there is a gradual phenotypic shift from haematological to neurological manifestations (Kearns-Sayre syndrome). Pearson syndrome is caused by large deletions of mitochondrial DNA (34), which are usually sporadic (congenital). Nearly half the patients have the “common deletion”, a 4977-base pair mtDNA deletion involving several genes encoding components of the mitochondrial respiratory chain (NADH dehydrogenase, cytochrome c oxidase, ATP synthase) as well as several mitochondrial transfer RNAs. **Figure 3** shows a new deletion of 3614 bp described by our group in a patient suffering from Pearson syndrome with severe SA (35). Profound mitochondrial dysfunction appears to be the cause of disturbed mitochondrial iron metabolism (for a suggested pathophysiological mechanism, see ref. (35)).

Figure 3: The mitochondrial genome, with large deletions of mtDNA typically found in Pearson syndrome



Red: a new 3614-bp deletion described by our group. From ref. (35) with permission.

3. Acquired sideroblastic anaemias

Acquired SAs can be classified into acquired *reversible* SA and acquired *clonal* SA (Table 2) (36). The reversible forms are attributable to drugs, toxins, or malnutrition, and are usually corrected by eliminating the offending agent. In contrast, acquired clonal SA is part of the phenotype of a clonal BM disease, which can remain clinically stable for many years but may sometimes undergo clonal evolution towards acute myeloid leukaemia.

3.1 Acquired reversible SA

3.1.1 Alcohol

Alcohol and its metabolite, acetaldehyde, can inhibit several steps of the haem synthetic pathway (36). Mitochondrial iron accumulation therefore seems to reflect an imbalance between the large amounts of iron imported into mitochondria for haem synthesis, and insufficient production of protoporphyrin IX to incorporate the iron.

Table 2: Acquired sideroblastic anaemias

Acquired reversible SA	Alcoholism Drugs (chloramphenicol, isoniazid) Copper deficiency (nutritional, zinc-induced, copper chelation)
Acquired clonal SA	Refractory anaemia with ring sideroblasts (RARS) Refractory anaemia with multilineage dysplasia and ring sideroblasts (RCMD) Refractory anaemia with ring sideroblasts and thrombocytosis (RARS-T)

The percentage of ring sideroblasts in the BM ranges from 10 to 70%, and the sideroblastic phenotype may be accompanied by marked vacuolisation of erythroid precursors. Siderocytes may be detectable in the peripheral blood by iron staining. Ring sideroblasts disappear from the BM within a few days to 2 weeks after withdrawal of alcohol. It takes somewhat longer to recover from the anaemia, particularly if alcohol consumption produced folate deficiency, too.

3.1.2 Drugs

Chloramphenicol

This antibiotic not only inhibits bacterial but also mitochondrial protein synthesis, via its direct action on the large ribosomal subunit of the organelle, thereby impairing the synthesis of respiratory chain subunits encoded by the mitochondrial genome. The resulting suppression of mitochondrial respiration may be aggravated by the fact that high-dose chloramphenicol also acts as an inhibitor of the complex I segment (i.e. NADH dehydrogenase) of the respiratory chain (37). Prolonged administration of chloramphenicol causes ineffective haematopoiesis with severe BM dysplasia, often including the formation of ring sideroblasts (38).

Antituberculous drugs

Isoniazid (INH) deprives ALAS2 of pyridoxal phosphate and therefore inhibits haem synthesis. This problem can be overcome by concomitant administration of pyridoxine (25 to 50 mg/d) (39). Pyrazinamid has also been implicated in causing reversible SA, probably through anti-vitamin B6 properties, too (40). However, a patient with acquired SA who had been given a four-drug combination including INH and pyrazinamide recovered on the withdrawal of isoniazid alone (41).

Other antibiotics

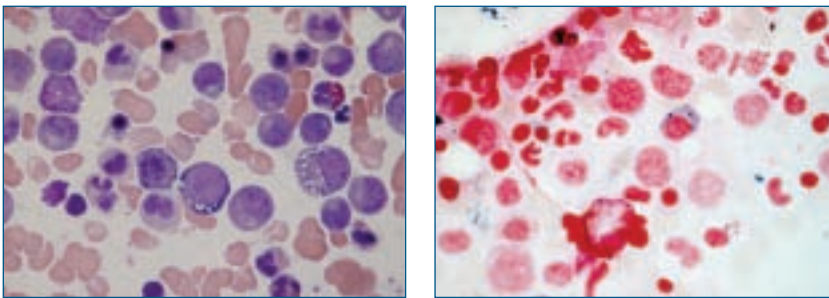
Lincomycin (42), fusidic acid (43) and linezolid (44, 45) have been implicated as causes of reversible SA but the relevant mechanisms of action are still unknown.

3.1.3 Copper deficiency

This condition has been described in patients receiving long-term parenteral or enteral hyperalimentation, after gastrectomy, with copper-chelating agents, or after excessive zinc ingestion. Copper deficiency can cause SA and mimic MDS (46, 47). Copper is an essential component of cytochrome c oxidase, i.e. complex IV of the mitochondrial respiratory chain. Therefore, copper deficiency can cause impairment of RS function (47) and may thus interfere with proper mitochondrial iron handling (see Section 2.5.2). The anaemia of copper deficiency can be accompanied by neutropenia with an absence of late myeloid forms in the BM. Typically, erythroid and myeloid precursors in the marrow are vacuolated (Figure 4). Surprisingly, in very severe copper deficiency in the experimental animal, mitochondria did not show iron overload (48). Iron remained in the cytoplasm, presenting as scattered ferritin molecules as well as ferritin molecules accumulating in siderosomes. Apparently, mitochondria were unable to import much iron through the inner membrane, probably due to insufficient mitochondrial membrane potential.

Copper deficiency can be induced by excessive zinc ingestion (example in Figure 4) because large quantities of ingested zinc interfere with intestinal copper absorption (49, 50). Oversupplementation of zinc is usually the cause, but reversible SA has also been reported as a consequence of zinc toxicity following ingestion of coins over a period of many years (51). Zinc must be discontinued for 9 to 12 weeks for full reversal of the anaemia and neutropenia (52).

Figure 4: A case of Wilson's disease treated with high doses of zinc who developed copper deficiency as a result of Zn intoxication



Characteristic vacuolisation of erythroid precursors was seen in the bone marrow (Wright's staining, left), and ring sideroblasts were also identified (Perls' staining, right) (patient diagnosed by Ph Beris).

3.2 Acquired clonal SA

3.2.1 Introduction

If non-neoplastic causes of SA have been excluded, the detection of ring sideroblasts in the BM strongly points to the presence of a MDS, particularly in elderly patients. The MDSs are a group of clonal haematopoietic stem cell diseases characterised by cytopenia(s), dysplasia in one or more of the major myeloid cell lineages, ineffective haematopoiesis, and increased risk of development of acute myeloid leukaemia (AML). In 1982, a French-American-British (FAB) Cooperative Group proposed a classification of MDSs which included refractory anaemia with ringed sideroblasts (RARS) among five MDS subtypes (RA, RARS, RAEB, RAEB-T, and CMML). The FAB classification has been widely used for more than two decades, not only because of its prognostic value, but also because it provided a basis for meaningful exchange of clinical and laboratory data between MDS working groups worldwide. In 2001, a WHO Classification was published which tried to amend some of the shortcomings of the FAB system (53). Very recently, the WHO Classification has been updated (54, 55). In order to foster a uniform nomenclature of MDS, this chapter on acquired clonal SA will draw on the new WHO Classification (2008). Patients with acquired clonal SA are included as one of three WHO-defined disease entities, namely RARS, RCMD(+RS) and RARS-T.

3.2.2 RARS

In the medical literature, BM disorders equivalent to RARS have usually been called acquired idiopathic sideroblastic anaemia (AISA), primary acquired sideroblastic anaemia (PASA), or pure sideroblastic anaemia (PSA). In the future, RARS should be the preferred term for this disease, which is morphologically and clinically confined to the erythroid lineage although the underlying defect involves a pluripotent haematopoietic stem cell. The following description of RARS is taken from the new WHO Classification (2008) (56).

Definition

RARS is a MDS characterised by anaemia, morphologic dysplasia in the erythroid lineage and ring sideroblasts comprising $\geq 15\%$ of the BM erythroid precursors. There is no significant dysplasia in non-erythroid lineages. Myeloblasts comprise $< 5\%$ of the nucleated BM cells and are not present in the peripheral blood (PB). Secondary causes of ring sideroblasts must be excluded.

Epidemiology

RARS accounts for approximately 3-11% of MDS cases. It occurs primarily in older individuals with a median age of 60-73 years and has a similar frequency in males and females.

Aetiology

Ring sideroblasts represent erythroid precursors with abnormal accumulation of iron within mitochondria, including some deposited as mitochondrial ferritin. Primary defects of haem synthesis (such as the delta-aminolaevulinic acid synthase defect in hereditary X-linked SA) can largely be excluded because protoporphyrin IX, the end product of porphyrin synthesis, is not decreased in RARS. Furthermore, acquired mutations in genes of the haem synthetic pathway have not been demonstrated in RARS. Therefore, a primary defect of mitochondrial iron metabolism is suspected. This defect may be caused by somatic mutations or deletions in nuclear or mitochondrial DNA. Clonality of CD34-positive progenitor cells and erythroid and granulocytic elements has been demonstrated in RARS patients by X-chromosome inactivation analysis. Stem cells from RARS patients display poor erythroid colony formation *in vitro* and manifest abnormal iron deposition at a very early stage of erythroid development. This evidence suggests that RARS represents a clonal stem cell defect that manifests as abnormal iron metabolism in the erythroid lineage and results in ineffective erythropoiesis.

Clinical features

The presenting symptoms are related to anaemia, which is usually of moderate degree; some patients may additionally be thrombocytopenic or neutropenic. There may be symptoms related to progressive iron overload.

Morphology

Patients typically present with normochromic macrocytic or normochromic normocytic anaemia. The red blood cells in the PB smear may manifest a dimorphic pattern with a major population of normochromic red blood cells and a minor population of hypochromic cells.

Blasts are not present in the PB. The BM aspirate smear shows an increase in the erythroid precursors with erythroid lineage dysplasia, including nuclear lobulation and megaloblastoid features. Granulocytes and megakaryocytes show no significant dysplasia (< 10% dysplastic forms). Haemosiderin-laden macrophages are often abundant. Myeloblasts are less than 5% of the nucleated BM cells. On an iron-stained aspirate smear, 15% or more of the red cell precursors are ring sideroblasts, as defined by 5 or more iron granules encircling one third or more of the nucleus. The BM biopsy is normocellular to markedly hypercellular, usually with a marked erythroid proliferation. Megakaryocytes are normal in number and morphology. Ring sideroblasts are frequently observed in other types of MDS. For example, cases with ring sideroblasts that have excess blasts in the PB or BM are classified as RAEB. When ring sideroblasts are 15% or more of the erythroid precursors but there are 10% or

more dysplastic cells in any non-erythroid lineage, and blasts are < 1% in the PB and < 5% in the BM with no Auer rods or monocytosis, the case is classified as refractory cytopenia with multilineage dysplasia (RCMD). Such patients have inferior survival to patients with RARS.

Immunophenotype

In RARS, aberrant immunophenotypic features of erythropoietic precursors can be found by flow cytometric analysis.

Genetics

Clonal chromosomal abnormalities are seen in fewer than 5-20% of cases of RARS and, when present, typically involve a single chromosome.

Prognosis and predictive factors

Approximately 1-2% of cases of RARS evolve to acute myeloid leukaemia. The reported overall median survival is 69-108 months.

3.2.3 RCMD

SA associated with multilineage dysplasia in the BM (RCMD+RS) was recognised as a separate type of MDS in the WHO Classification of 2001. The new WHO Classification (2008) incorporates these cases into the RCMD category because RCMD+RS has a clinical course indistinguishable from RCMD. The following description of RCMD is taken from the new WHO Classification (2008) (57).

Definition

Refractory cytopenia with multilineage dysplasia (RCMD) is a type of MDS with one or more cytopenias and dysplastic changes in two or more of the myeloid lineages: erythroid, granulocytic, megakaryocytic. There are < 1% blasts in the PB and < 5% in the BM. Auer rods are not present and the monocytes in the PB are less than $1 \times 10^9/L$. The recommended levels for defining cytopenias are haemoglobin < 10 g/dL, absolute neutrophil count < $1.8 \times 10^9/L$, and platelet count < $100 \times 10^9/L$. However, values in excess of these thresholds are not exclusionary of a diagnosis of MDS if definitive morphologic and/or cytogenetic findings are consistent with a diagnosis, e.g. complex cytogenetic abnormalities. The thresholds for dysplasia are $\geq 10\%$ in each of the affected cell lines. In assessing dysplasia it is recommended that 200 neutrophils and precursors and 200 erythroid precursors be evaluated in smear and/or trephine imprint preparations. The neutrophil dysplasia may be evaluated in PB or BM smears. At least 30 megakaryocytes should be evaluated for

dysplasia in BM smears or sections. In some cases, dysplastic megakaryocytes may be more readily identified in sections than smears. In particular the presence of micromegakaryocytes should be noted. Cases with multilineage dysplasia and 2-4% blasts in the PB, > 5% in the BM, and no Auer rods should be classified as RAEB-1; cases with 1% blasts or fewer in the PB and < 5% blasts in the BM, and Auer rods should be classified as RAEB-2; cases with 1% blasts in the PB and < 5% in the BM and no Auer rods should be classified as MDS-U. Some cases of RCMD have $\geq 15\%$ ring sideroblasts.

Morphology

For details, see (55) and (57). In RCMD, variable numbers of ring sideroblasts may be identified.

Genetics

Clonal cytogenetic abnormalities including trisomy 8, monosomy 7, del(7q), monosomy 5, del(5q), and del(20q), as well as complex karyotypes, may be found in up to 50% of patients with RCMD.

Prognosis and predictive factors

The clinical course varies. Most patients with RCMD have International Prognostic Scoring System (IPSS) scores in the intermediate category. Prognostic factors relate to the degree of cytopenia and dysplasia. The frequency of acute leukaemia evolution at two years is ~10% in RCMD. The overall median survival is approximately 30 months. Patients with complex karyotypes have survivals similar to patients with RAEB.

3.2.4 RARS-T

Refractory anaemia with ring sideroblasts associated with marked thrombocytosis (RARS-T) is a "myelodysplastic/myeloproliferative neoplasm, unclassifiable" according to the new WHO Classification. Until recently, the diagnosis required at least 15% ring sideroblasts in the BM and a persistent platelet count of at least $600 \times 10^9/L$ in the peripheral blood. In the latest version of the WHO Classification (58), the cut-off value for thrombocytosis was decreased to $450 \times 10^9/L$, which is lower than in the majority of published articles that generally used the higher cut-off level. RARS-T is probably the first MDS "subtype" to reveal the cause of its growth advantage: about 60% of patients with RARS-T carry the JAK2 V617F mutation (59-68), which is characteristic of myeloproliferative syndromes. The following description of RARS-T is taken from the new WHO Classification (2008) (58).

Definition

In the third edition of the WHO Classification, RARS-T, previously also referred to as essential thrombocythaemia (ET) with ring sideroblasts, was proposed as a provisional entity to encompass patients who have the clinical and morphological features of the MDS, RARS, but who also have marked thrombocytosis associated with abnormal megakaryocytes similar to those observed in the BCR-ABL1 negative MPN, such as ET or early-stage primary myelofibrosis (PMF). However, some investigators have suggested that RARS-T is not a unique entity but instead represents cases of other subtypes of MDS or well-defined MPN that have acquired ring sideroblasts as a secondary form of dysplasia. It is not clear whether RARS-T is a distinct entity, one end of the spectrum of RARS, a progression of RARS due to an additional acquired genetic abnormality, or less likely, the occurrence of two rare diseases in the same patient. Therefore, until these questions are more clearly answered, RARS-T remains a provisional entity.

In support of a myeloproliferative component to this neoplasm, the majority of cases reported as RARS-T have shown the JAK2 V617F mutation, or much less commonly, the MPL W515K/L mutation. On the other hand, the few reported cases with this mutation that have been studied for endogenous colony formation *in vitro* have demonstrated a pattern more akin to that of MDS. Thus it may be that the provisional designation of an MDS/MPN accurately reflects the underlying biology in a substantial proportion of the patients; more study is needed to further clarify this disorder.

Cases with isolated del(5q), t(3;3)(q21;q26) or inv(3)(q21;q26) are excluded from this category, as are cases with a BCR-ABL1 fusion gene. In addition, if there has been a prior diagnosis of an MPN without ring sideroblasts, or there is evidence that the ring sideroblasts might be a consequence of therapy or represent disease progression in a patient with features that meet the criteria of another well-defined MPN, this designation should not be used.

Morphology

These cases have features of RARS (anaemia with no blasts in the PB and dysplastic, ineffective erythroid proliferation often with megaloblastoid features, ring sideroblasts $\geq 15\%$ of the erythroid precursors, and $< 5\%$ blasts in the BM) and thrombocytosis with a platelet count $\geq 450 \times 10^9/L$ associated with proliferation of large atypical megakaryocytes similar to those observed in BCR-ABL1 negative MPN. The minimum platelet count required for inclusion has been lowered to $450 \times 10^9/L$ from $600 \times 10^9/L$ for consistency with the defining criterion for ET, and because several studies have demonstrated that patients with platelet counts lower than $600 \times 10^9/L$ may have biological features, including JAK2 V617F mutations,

similar to those with counts $\geq 600 \times 10^9/L$. It is important to note that the criteria for RARS-T include morphologically abnormal megakaryocytes similar to those observed in ET and in PMF. This criterion should aid in distinguishing RARS-T from those cases of RARS commonly reported to have a modest increase in their platelet count. Nevertheless, we recommend testing for JAK2 V617F when the platelet count is elevated in patients with RARS until the borderline between RARS and RARS-T is more clearly defined.

Genetics

The recent discovery that up to 60% of patients with RARS-T harbour the JAK2 V617F mutation (an incidence similar to that found in ET and PMF) or less commonly, the MPL W515K/L mutation, not only elucidates the reason for the proliferative aspect of RARS-T but also would seem to move it closer to the MPN category. Thus, studies for JAK2 V617F, and, if indicated, for the MPL W515K/L mutation should always be performed in such cases.

3.2.5 Pathophysiological considerations in acquired clonal SA

Prevalence of the sideroblastic phenotype in MDS

The presence of ring sideroblasts is a common morphological change in MDS and may therefore be closely connected with a basic pathophysiological mechanism of myelodysplasia. Ring sideroblasts are not confined to RARS but were also found in refractory anaemia (RA), refractory anaemia with excess of blasts (RAEB), and even in some cases of RAEB-T according to the FAB Classification (69, 70). Jacobs and Bowen (71) stated that in refractory anaemia "the number of erythroblasts with ring siderotic granules may vary from 1 to 90% and there is no clear demarcation between 'sideroblastic' and 'non-sideroblastic' cases".

Electron microscopy is more sensitive than light microscopy for detecting mitochondrial iron overload as well as other mitochondrial changes in MDS (72-74). For example (75), ultrastructural characteristics of erythroblasts were investigated in 22 patients, only two of whom had been diagnosed as SA (RARS according to FAB) on light microscopy. Nevertheless, 16 patients (73%) showed iron-laden mitochondria. In 55% of the cases, the mitochondria were enlarged with or without disruption of internal cristae and/or mitochondrial membranes, which was significantly associated with accumulation of iron. In all patients, erythroblasts showed extensive cytoplasmic vacuolisation. The latter finding is also characteristic of erythroblasts in patients with Pearson syndrome (see section on hereditary SA of this Chapter), which is caused by large deletions of mitochondrial DNA (76).

Proposed mechanism of mitochondrial iron accumulation

In acquired clonal SA, a primary defect in the haem biosynthetic pathway is very unlikely because the penultimate product of this pathway, protoporphyrin IX, is elevated rather than reduced (77). The defect can therefore be narrowed down to the last step of haem biosynthesis, i.e. the insertion of iron into protoporphyrin IX by ferrochelatase. Although impaired ferrochelatase activity was found in approximately one half of patients studied (78), another group reported that increased red cell protoporphyrin concentrations were not correlated with low ferrochelatase activities (79). Furthermore, Steensma et al. (80) performed a candidate gene mutation analysis and found no somatic missense mutations of the ferrochelatase gene and its promoter in patients with acquired idiopathic SA. While decreased ferrochelatase activity may represent a secondary effect of mitochondrial iron overload, it may also be a direct consequence of impaired mitochondrial iron-sulfur cluster biosynthesis, since ferrochelatase contains a C-terminal [2Fe-2S] cluster which is essential for its function (81).

However, impaired insertion of iron into protoporphyrin IX may also result from a mechanism that is not attributable to a ferrochelatase defect. Several examples, like copper deficiency, chloramphenicol toxicity, and mitochondrial dysfunction due to mtDNA deletions (Pearson syndrome (76)), suggest that erythroid precursors can develop mitochondrial iron accumulation as a consequence of impaired mitochondrial respiration. **Figure 2** illustrates the hypothetical link between respiratory chain dysfunction and the sideroblastic phenotype.

The initial evidence of RS dysfunction in the BM of MDS patients was provided by Aoki (82), who examined several mitochondrial enzymes in 61 patients with primary acquired SA. Cytochrome c oxidase (COX) and oligomycin-sensitive ATPase, both components of the respiratory chain, had reduced activity compared with controls, whereas citrate synthase, an enzyme of the mitochondrial matrix, was not impaired. The reduced activity of COX and oligomycin-sensitive ATPase was not secondary to excess mitochondrial iron, because enzyme measurements were performed using the patients' granulocytes, which do not show mitochondrial iron loading. Every case of primary acquired SA had decreased COX activity in mature granulocytes.

Matthes et al. observed decreased mitochondrial membrane potential in erythroblasts of patients with RARS (83), but later found no biochemical evidence of COX deficiency in BM homogenates from five patients with RARS (35).

Bowen et al. (84) measured oxygen consumption by peripheral blood mononuclear cells (MNCs) from patients with MDS and healthy controls. Oxygen consumption by MNCs from MDS patients was significantly decreased, pointing to a defect in mitochondrial respiration.

Mitochondrial dysfunction may inhibit iron utilisation in erythroblasts even in the absence of a sideroblastic phenotype. If respiratory chain activity is heavily compromised, as in severe experimental copper deficiency, the membrane potential of erythroblast mitochondria may become insufficient to support the import of iron into the mitochondrial matrix. This would preclude the expression of the sideroblastic phenotype.

In vitro, erythroid progenitor cells from low-risk MDS spontaneously release cytochrome c from mitochondria, resulting in activation of capase-9 and subsequent cell death (85). Apparently, defective mitochondria trigger or favour the execution of apoptosis in MDS BM cells, especially erythroid cells.

Possible causes of mitochondrial dysfunction in acquired clonal SA

Mitochondrial dysfunction can be caused by mutations of mitochondrial DNA (mtDNA) because several subunits of the mitochondrial respiratory chain are encoded in the mitochondrial genome. The frequency and spectrum of somatic mtDNA mutations in the BM of patients with MDS was recently determined using heteroduplex analysis with denaturing HPLC (86, 87). The analysis included 104 patients with MDS (24 RA, 32 RARS, 34 RAEB, 7 RAEB-T, 7 CMML), 3 patients with AML from MDS, and 36 patients with myeloproliferative disease (23 CML, 9 PV, 4 IMF). Heteroplasmic mtDNA mutations, mostly transitions, were identified in 56% of MDS and 44% of MPD patients. In MDS, mutation frequency increased with age and more advanced disease. Mutational spectra showed no hot spots and were similar in different types of MDS. Heteroplasmic mutations generally did not represent known polymorphisms. About half of them affected conserved amino acids or nucleotides. Mutations were less frequent in protein encoding genes (50x10⁶ base pairs) than other mitochondrial genes (transfer RNAs, ribosomal RNAs and control region; about 80x10⁶ base pairs). The study thus yielded a high frequency of acquired, clonally expanded mtDNA mutations in MDS. However, the functional importance of these mutations remains unclear, considering that genotype correlates poorly with phenotype in mitochondrial diseases. A previous search for mtDNA mutations in 10 cases of MDS, performed by Shin et al. (88), revealed some homoplasmic sequence alterations but, surprisingly, no heteroplasmic mutations. This may be due to the fact that mutation scanning was based on direct DNA sequencing, which is less well suited for detection of heteroplasmy, i.e. coexistence of mutant and wild-type mtDNA.

Despite their possible contribution to the sideroblastic phenotype, mtDNA mutations cannot be the sole cause of acquired clonal SA, because they are unlikely to provide the cellular growth advantage that is necessary for clonal expansion.

However, a stem cell harbouring mutant mtDNA may incur additional mutations in nuclear DNA which then confer an abnormal cellular growth advantage.

Mutations in nuclear DNA may not only drive clonal expansion but may also be responsible for mitochondrial dysfunction resulting in an MDS-like picture, as exemplified by mutational loss of HSPA9B in zebrafish (89, 90). HSPA9B, encoded in the critical deleted region of chromosome 5, is a highly conserved, ubiquitously expressed mitochondrial chaperone that is important for the transport, folding, and assembly of mitochondrial matrix proteins.

Regarding mitochondrial defects producing the sideroblastic phenotype, it has been shown that increased expression of mitochondrial ferritin (MtF) is an early event in the development of mitochondrial iron accumulation (4, 91). This feature can be utilised for diagnosing SA by flow cytometry (92). However, the increase in MtF is probably a secondary phenomenon. This is suggested by the association of mitochondrial ferritin with cell types characterised by high metabolic activity and oxygen consumption, which indicates a role in protecting mitochondria from iron-dependent oxidative damage (93). Furthermore, over-expression of mitochondrial ferritin was shown to cause cytosolic iron depletion (94), which is not observed in SA.

Finally, many features of acquired clonal SA could be explained by a primary defect of mitochondrial iron-sulfur cluster biogenesis (95), which could impair, among other cellular functions, ferrochelatase and respiratory chain activity. Recently, the ABCB7 transporter, which is thought to transport an essential component of Fe/S protein production from mitochondria to the cytosol, was found to have uniformly reduced transcript levels in 33 patients with RARS (96). However, it is not known whether the level of the ABCB7 protein is altered, and the finding may also represent a secondary effect.

3.2.6 Treatment of acquired clonal SA

RARS

Treatment of RARS and RCMD+RS has recently been summarised in a review (97). Immuno-suppressive treatment and thalidomide, which may be efficacious in subsets of RA, are less successful in RARS (98). Lenalidomide abrogated transfusion need in around 25% of patients with erythropoietin (Epo)-refractory RARS, with a median duration of response of 43 weeks. While several reports have shown that the response rate to Epo in RARS is lower than the response in RA or early RAEB, the effect of the combination of Epo and granulocyte-colony stimulating factor (G-CSF) is better in RARS than in other subsets of MDS, with an overall and complete erythroid response rate of 50% and 38%, respectively (99). According to a recent

report, the survival of patients with RARS (WHO) treated with Epo+G-CSF was 121+ months, with a response rate of 71% and a response duration of 28 months (100). No patient with RARS (WHO) developed AML.

RCMD

RCMD(+RS) patients showed a lower response rate (30%) to Epo+G-CSF, but a similar response duration (25 months, range 27-95) and shorter survival (31 months, range 14-43). Among the RCMD(+RS) patients, 10% developed AML. Altogether, treatment with Epo+G-CSF in patients with MDS and ring sideroblasts is safe and leads to prolonged responses, particularly in patients with no or low transfusion need (100).

RARS-T

There are no specific treatment recommendations for RARS-T. Patients with RARS-T (platelets > 450×10⁹/L) do not differ from anaemic patients with RARS or RCMD-RS regarding response to treatment with Epo+G-CSF (97). An increased risk of thromboembolic complications has not been reported for RARS-T. In case of severe thrombocytosis, it is reasonable to treat RARS-T like ET.

Treatment of iron overload

Most patients with acquired clonal SA will become transfusion-dependent during the course of their disease. At the same time, most of them belong to the low or intermediate-1 risk categories of MDS, according to the IPSS (101), and can therefore expect to survive for several years. Accordingly, these patients are at risk of developing transfusional iron overload. There is accumulating evidence that iron overload in MDS patients is associated with decreased life expectancy (102, 103), and that chelation therapy has a favourable effect on prognosis (104, 105).

Intensive treatment with deferoxamine can achieve a negative iron balance in MDS and can even improve erythropoietic output (106). However, parenteral chelation therapy with deferoxamine is cumbersome. The more convenient oral iron chelator deferasirox is also effective in MDS, with manageable side effects (107-109).

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Multiple Choice Questionnaire

To find the correct answer, go to <http://www.esh.org/iron-handbook2009answers.htm>

1. Which of the following forms of sideroblastic anaemias is *not* inherited in an autosomal recessive manner?

- a) Glutaredoxin-5 deficiency
- b) ALAS2 deficiency
- c) Thiamine-responsive megaloblastic anaemia
- d) None of the above

2. Pearson syndrome has which of the following features:

- a) Severe transfusion-dependent sideroblastic anaemia
- b) Exocrine pancreatic insufficiency
- c) Metabolic acidosis with high blood lactate
- d) All of the above

3. Acquired reversible sideroblastic anaemia can be due to which one of the following?

- a) Zinc deficiency
- b) Copper deficiency
- c) Vit B12 deficiency
- d) Iron overload

4. Which one of the following statements about acquired clonal sideroblastic anaemia is correct?

- a) Ring sideroblasts are found in different types of the myelodysplastic syndrome
- b) Mitochondrial iron overload in acquired clonal SA is attributable to mutations in erythroid delta-aminolaevulinic acid synthase (ALAS2).....
- c) In acquired clonal SA, mitochondria are characterised by a deficiency of mitochondrial ferritin
- d) Ferrochelatase is mutated in the majority of patients with acquired clonal SA

5. Which one of the following statements is correct?

- a) Patients with acquired clonal SA usually respond to treatment with Vit B6 ..
- b) Patients with RARS have a higher reponse rate to Epo than other types of MDS
- c) The response rate to Epo+G-CSF in patients with RARS (WHO) is about 70%
- d) Patients with RARS do not benefit from iron chelation therapy because their life expectancy is too short.....