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Constitutional Hypoplastic Anemia

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ABSTRACT A large number of patients seen in the hematological practice belong to the broad group of bone marrow failure syndromes (BMFS) - comprising of aplastic anemia (AA) and myelodysplastic syndromes (MDS), AA being much more frequent than MDS. AA is diagnosed in the presence of hypocellular bone marrow and peripheral blood cytopenias. This disorder may be constitutional or acquired. The constitutional disorder may be either congenital (present at birth) or inherited (genetic but not necessarily expressed at birth). Multiple etiologies are implicated (Table 1) and the natural history of AA patients is very diverse (Alter 1993; Alter and Young 1993). According to the western literature, constitutional factors are operative in at least 1/3 of young patients with BMFS (Alter and Young 1993). The definition of exact etiology is important for the obvious reasons of proper management, family screening and genetic counseling etc.

FANCONI'S ANEMIA

Acquired primary type of AA is reported to be the more common variety. However this diagnosis should only be made as such, in the absence of secondary and constitutional etiological factors[2,9]. Of these, FA will be described here in greater detail, since it is the most common constitutional factor responsible for BMFS. FA, an autosomal recessive disorder, is the best defined but perhaps most enigmatic of the inherited disorders of bone marrow failure[1,2,16]. It is characterized by a progressive pancytopenia, various conge-nital abnormalities and increased predisposition to malignancy. The physical phenotype includes spectrum from extremely abnormal to normal, the hematological findings range from severe aplastic anemia to normal and the malignant potential includes leukemia, carcinomas, liver tumours or no malignancy[1-3].

Although more than 90% of FA patients are presumed to develop aplastic anaemia at some point in time, there seems to be a temporal continuum, whereby aplasia occurs early, leukemia later and solid tumours even later[1-3].

Historical Perspective

Fanconi in 1927, described 3 brothers with aplastic anemia and congenital physical abnormalities. Other reports contributed to the expansion of the phenotype. In 1944, Dacie and Gilpin reported 3 brothers, one each with the diagnostic label of FA, leukemia and paroxysmal nocturnal hemoglobinuria (PNH); although the common link obviously was not recognized. Nearly 20 years later, Estren and Dameshek described 2 families in whom several siblings had AA but physical appearance was normal (barring short stature). Thirty more years elapsed before these two disease entities, FA and 'Estren and Dameshek type of constitutional aplastic anemia' were recognized to belong to the same disease spectrum. This was followed by numerous reports of families in which 'classical' FA was diagnosed in one sibling whereas other sibling(s) had aplastic anemia without congenital malformations. The cases published till 1980's, as defined on the basis of hematological and physical manifestations, reveal a bias towards the more severe clinical cases[1,2]. International Fanconi Anemia Registry (IFAR) was established in 1982, at the Rockefeller University. The concerted efforts of IFAR have generated very interesting database on the clinical, hematological and genetic parameters of FA patients; thus highlighting the entire spectrum of diverse features of this syndrome[8]. The phenotypic heterogeneity of FA is more widespread, as revealed by IFAR data, than was previously understood. Both physical and hematological abnormalities are detected in 1/3, only physical abnormalities in 1/3 and only hematological abnormalities are present in rest of the patients[1,3,8].

Clinical Features

There is great variability in the clinical features of FA patients and may involve almost every organ system[1,3,8]. The characteristic physical features, when present, may help in bringing the patient earlier to the clinician. Typical clinical features of FA are shown in Table 2.

Table 1: Etiological classification of Aplastic Anemia

- Constitutional
  - Fanconi's anemia (FA)
  - Dyskeratosis congenita (DC)
  - Shwachman - Diamond syndrome (SD)
  - Amegakaryocytic thrombocytopenia
  - Familial aplastic anemia
- Acquired
  - Idiopathic
  - Secondary
    - Infections
    - Toxic exposure
    - Thymoma
    - Pregnancy
    - Immunological diseases
    - Paroxysmal nocturnal hemoglobinuria (PNH)

Such a wide spectrum of clinical features lends support to the notion that FA genes play an important role in the basic developmental process.

Hematological Heterogeneity

Similar to the aforementioned features, the type and degree of hematological involvement is also quite varied. Pancytopenia usually develops during the first decade of life (the average age of onset of AA is 8 years) and death results from its complications. Thrombocytopenia and anemia appear early, followed by neutropenia. Signs of stress erythropoiesis are displayed in the form of expression of fetal hemoglobin, macrocytosis, i antigen and high serum erythropoietin[1,2]. The patients (even within a single family) may belong to different categories of hematological impairment: group 6 - normal blood counts; group 5 - mild marrow dysfunction; group 4 - needing treatment; group 3 - responding to treatment; group 2 - failing treatment; and group 1 - failed treatment[1].

The bone marrow findings, true to the concept of variability, may not correlate with the above mentioned hematological impairment groups, with the exception of patients in groups 1 and 2. Bone marrow cellularity may range from severely hypoplastic, normal, myelodysplastic, or even hypercellular. According to the IFAR data, the actuarial risk of developing pancytopenia and MDS / AML was 98% and 52% at 40 years of age, respectively[1-3]. Clonal cytogenetic abnormalities have been described in marrow samples of FA, although their significance is not yet clearly defined. Sequential development of hematological abnormalities most likely occurs as a result of absence of FA gene product per se, or in combination with other extrinsic or intrinsic potentially leukemogenic factors. FA serves as an interesting model of leukemogenesis as these patients would eventually develop MDS and / or AML, provided they survive long enough[1-3].

Other Late Complications

FA patients are at a high risk for development of malignancies[1-3]. Though the risk for MDS / leukemia is very well known, it is less commonly appreciated about other malignancies. Since there has been a distinct improvement in the life expectancy of FA patients over the years (10 years in 1960s to 30 years in 1990s) the risk of these complications is ever increasing

Table 2: **Frequency of physical features in FA**

Organ system	*Specific abnormalities*	*Frequency (%)*
Skin	hypo- and hyperpigmentation, café au lait spots	62
Short stature	–	59
Upper limbs	thumbs, radii, hands	48
Hypogonadism	13:1 male: female	42
Head	microcephaly, micrognathia	26
Eyes	small epicanthal folds, hypertelorism, nystagmus, strabismus	25
Renal	ureter and kidney anomalies, reflux	24
Low birthweight	less than 2500 g	14
Retardation	–	13
Lower limbs	feet, toes, hip dislocation, osteoma	10
Ears	deafness, low set, dysplastic, middle ear defects	10
Other	hyperreflexia, congenital heart defects, gastrointestinal atresia, tracheoesophageal fistula, Meckel's diverticulum, imperforate anus.	<9

as the patients move in to adulthood, particularly those who have had bone marrow transplantation or gene therapy. Liver cancers reportedly develop in at least 5% and tumours involving oropharynx, gastrointestinal tract and gynecologic systems in another 5%[2]. The treatment unfortunately remains difficult, the reason being too narrow a therapeutic index for chemotherapy or radiation, due to the basic defect of DNA instability.

Chromosomal Breakage Studies

Several workers in early 1960's observed increased chromosomal breakage in patients with FA, however spontaneous breakage in chromosomes from peripheral blood lymphocytes was found to be an insensitive and nonspecific feature. The hypersensitivity to clastogenic effect of DNA cross linking agents as a unique marker for FA was reported in 1970's[1,2]. The patients with increased chromosomal breakage on exposure to DNA cross linking agents such as diepoxybutane (DEB), mitomycin C (MMC) and nitrogen mustard are now considered to have FA, whereas patients with any or every symptom and sign that is considered "classic", are diagnosed as non-FA, in the absence of increased chromosomal breakage[1,8]. Various laboratories use more than one clastogenic agents for diagnosing FA, however IFAR study group experience shows DEB to be a more reliable agent than others. Distinct clinical differences were demonstrated between DEB -ve and DEB +ve patient groups, which further supports the concept that DEB sensitivity is a reliable marker for FA diagnosis[8]. In fact IFAR study group has presented a simplified scoring system based upon eight clinical features that are best predictors of DEB positivity. These include growth retardation, birth marks, kidney and urinary anomalies, microphthalmia, learning disabilities, low platelets, thumb and radius anomalies and others. The cellular characteristic of hypersensitivity to DEB can be used for prenatal as well as postnatal diagnosis. The study of DEB sensitivity is extremely important for the differential diagnosis of aplastic anemia and various syndromes with congenital abnormalities. However the cellular heterogeneity of FA is also well appreciated by now. There have been reports of patients whose chromosomal breakage rate varied with time and others in whom only a small clone of cells showed breaks at a given time[2,8]. Other shortcomings of DEB test are - its inability to distinguish carriers of FA from normal persons and diagnose double heterozygote with mosaicism; in addition to poor correlation with clinical severity[16]. However DEB remains the gold standard test for detection of FA defect in suspected patients[8].

Molecular Variability

Keeping the clinical, hematological and cytogenetic heterogeneity of FA in mind, it is obvious that FA is a genetically heterogeneous disorder and the right answers would be available only by using molecular biological approach. Involvement of more than one gene in the causation of FA was deduced with the use of somatic cell hybridization studies which assign the patients into different complementation groups; the hybrid cells of different complementation groups correct the chromosomal breakage where as hybrid cells from the same group still show chromosomal breakage[7]. So far eight complementation groups of FA (FA-A, FA-B, FA-C, FA-D, FA-E, FA-F, FA-G, FA-H) have been identified and are likely to represent eight individual genes responsible for FA phenotype[13]. Analysis of FA complementation groups by cell hybridization studies is dogged by certain inherent technical limitations which makes its large scale screening application less suitable. Therefore workers have tried to circumvent this problem by mapping and cloning different FA genes. Thereafter screening for specific mutations in particular FA genes followed. So far genes for FA-C and FA-A have been cloned and mapped to chromosome 9q3.22 and 16q24.3 respectively, while FA-D gene has been mapped to chromosome 3p22.26. There are 43 exons in FA-A gene and 14 exons in FA-C gene. As of now, the molecular analysis of only FA-C[9,10,22,23] and FA-A[24] has been reported.

Very few studies have been published detailing the diagnosis of FA subgroups from various parts of the world. FA-C is reported to be very prevalent in Ashkenazi Jews[22,23], FA-A in South African Afrikaan-speaking people[17]

and Italians[8] A small sample of 21 FA patients from Germany and Netherlands however, revealed all eight complementation groups[13]. All these studies emphasize the geographic variation of different FA subgroups. Some FA genotypes correlate with the phenotypic variation of the disease. FA-C patients of Jewish ancestry with IVS 4+4 A→T mutation have more severe disease with profound congenital abnormalities and early compromise of hemopoiesis, whereas disease runs a milder course in patients with 322 del G mutation[10,21]. Such correlation of genotype and phenotype has not emerged in FA-A as yet because of heterogenous nature of molecular lesions[24]. Such phenotypic corre-lation would go a long way to guide therapeutic options of patients with severe FA or otherwise. IFAR study group has stressed the need for timely and correct diagnosis in the pre anemic phase (Giampietro et al. 1993). Molecular studies for mutation analysis can be performed once the diagnosis of FA has been established by DEB sensitivity.

Cellular Phenotype

In addition to cross linking agent (like DEB) sensitivity, several other phenotypic abnormalities have been described in FA cells[6]. These include prolongation of G2 phase of cell cycle, sensitivity to oxygen and ionizing radiation, overproduction of tumour necrosis factor - alpha, increased apoptosis, defective p53 induction and intrinsic stem cell defect etc. Most of these abnormalities are possibly epiphenomena and their relationship to the primary FA defect is not clearly understood[6].

Frequency of FA

Though a rare autosomal recessive disorder, FA is the most common cause of constitutional BMFS, with an estimated frequency of 1 per 360000 births[16]. The estimated heterozygote frequency has been reported to vary between 1/ 200 to 1/300 in certain populations from the west [1,16]. Hou and Wang (1997) from Taipei, have reported detection of 3 FA patients out of 33 BMFS cases, on the basis of MMC induced chromosomal breakage (DEB did not work). However no published data is available from India, as the specific tests remain out of the reach of most of our AA patients. Aplastic anemia affects younger patients more frequently in the orient. In the studies conducted at our institute, nearly 40-50% of aplastic anemia patients were found to be younger than 20 years of age and only 12% above 50 years of age[5,20]. This is very much different compared to the reports from the west, which have shown 80% of cases above 50 years[4] and 40% patients above 60 years of age (International Agranulocytosis and Aplastic Anemia Study, 1987)[12]. This strongly emphasizes the need to suspect constitutional factors in our AA patients. We have detected FA defect in 10/35 (28.5%) aplastic anemia patients - 25 pediatric and 10 young adults (Proceedings of 41st Annual Conference of ISHTM, Mumbai 2000).

Diagnosis of FA and Its Implications

FA would remain an under diagnosed entity and it would be difficult to estimate the real incidence unless the appropriate tests (DEB induced chromosomal breaks) are applied consistently over a wide range of age groups, in patients with BM failure syndromes, with or without physical abnormalities[1,2,8] .

A diagnosis of FA should be considered in young adults as well as children with bone marrow failure, as 10% of the FA patients reported in the literature were found to be at least 16 years of age at the time of diagnosis, although some of the patients might present much later with varied manifestations e.g. acute myeloid leukemia[1,2,16]. An accurate diagnosis is important in these patients from the point of therapeutic decision making and to provide genetic counseling. FA patients have a high rate of response to androgen therapy whereas non-FA aplastic anemia patients respond to treatment with anti-thymocyte globulin (ATG) and/or cyclosporin A. The correct diagnosis is also important in relation to bone marrow transplantation and chemo-therapy. Because of hypersensitivity to all DNA cross linking agents, these patients require a modified regimen with less than usual dose of chemo- and/or radiotherapy[1,2,8].

Dyskeratosis congenita (DC) is a related but rare BM failure syndrome, characterized by early onset of abnormal skin pigmentation, nail dystrophy and mucosal leukoplakia. The inheritance pattern is X -linked in >90%, autosomal dominant or autosomal recessive in

the rest. Progressive BM failure develops in more than 40% patients by the age of 10 years and more than 80% by the age of 30 years. Skin fibroblasts and bone marrow cells of DC patients show chromosomal instability[14,15].

Shwachman - Diamond (SD) syndrome is characterized by exocrine pancreatic insufficiency and neutropenia. Signs of pancreatic insufficiency are detected in early infancy. Neutropenia, associated with skin infections and pneumonia, appears in infancy or early childhood. Other important findings include malnourishment and short stature (50%), mental retardation (15%), anemia (15%), thrombocytopenia (7%) or both (20%). SD syndrome patients also have propensity for malignancies[2].

Amegakaryocytic Thrombocytopenia

A minority of constitutional aplastic anemia patients present with thrombocytopenia in infancy, and pancytopenia supervenes later. This needs to be differentiated from the thrombocytopenic disorders with increased bone marrow megakaryocytes and congenital viral (and other) infections. A variety of physical abnormalities have been reported in the literature, some resembling those found in FA. However FA can not be excluded in these cases on a retrospective basis, without the help of DEB test[2].

Familial Aplastic Anemia

A group of so called familial aplastic anemia cases do not fit into any of the well described entities. This has to be a diagnosis of exclusion[2].

In the present time, FA truly represents a cancer susceptibility syndrome with great heterogeneity in its clinical, cellular and molecular characteristics, and possesses a high penetrance rate for aplastic anemia, leukemia and solid tumours[1,2,3]. It is a challenging task to diagnose FA patients early and manage them properly; in addition to undertaking family screening and genetic counseling. With the availability of advanced techniques, cellular and molecular heterogeneity of FA (and other constitutional aplastic anemias) hopefully will be unraveled much sooner than later.

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