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13th Floor, New Multistoreyed Building, K.E.M. Hospital Campus, Parel, Mumbai - 400 012. (INDIA) web : www.niih.org.in

IMMUNOHAEMATOLOGY BULLETIN

Bulletin of The National Institute of Immunohaematology
(I.C.M.R.)

Vol. 44; No. 1 Jan- April 2013

CYTOGENETIC AND MOLECULAR CHANGES IN MYELODYSPLASTIC SYNDROMES

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Summary

Myelodysplastic syndromes (MDS) are characterized by ineffective hematopoiesis and a high propensity to transform to acute myeloid leukemia (AML). The cytogenetic changes and gene mutations play an important role in the pathogenesis of the disease. The latter have been integrated into prognostic scoring systems including the IPSS (International Prognostic Scoring System) and WPSS (World Health Organization [WHO] classification-based Prognostic Scoring System). In these systems and in multivariate analysis comparing clinical and genetic data, complex karyotypes are associated with a particularly poor prognosis. 5q deletion plays a distinct role by classifying the only genetically defined WHO subtype. Also, due to advancement in technology such as whole genome sequencing, the number of known mutations occurring in MDS is steadily increasing. Recent discoveries include mutations in EZH2, DNMT3A, ASXL1 and IDH1/2. Like TET2, the most commonly mutated gene in MDS, all are involved in epigenetic regulation. Mutations such as ASXL1, RUNX1 EZH2, ETV6/TEL and TP53 have an adverse impact on patient overall survival. Early evidence suggests that some mutations might influence treatment response, necessitating reassessment of the prognostic effect of genetic alterations in the light of every new treatment. This review discusses clinical and biological effects of the most common cytogenetic and molecular aberrations in patients with MDS.

Introduction

Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal hematopoietic stem cell diseases characterized by cytopenia(s), dysplasia of one or more lineages, ineffective hematopoiesis and an increased risk of transformation into acute myeloid leukemia (AML) [1]. Clinically, MDS presents with (transfusion-dependent) anemia, infections due to neutropenia and/or bleeding due to thrombocytopenia. MDS is a disease of the elderly with a median age of 70 years, and an incidence of 3 – 5/100 000 persons rising to >20/100 000 among those over 70 years [1]. In recent years, it has become evident that genetic markers provide significant prognostic information for patients with MDS. The well-established International Prognostic Scoring System (IPSS) includes clinical, morphologic and cytogenetic information differentiating between good-, intermediate- and poor-risk groups (Table 1) [2]. The new World Health Organization (WHO) classification-based Prognostic Scoring System (WPSS) attributes an even higher risk to patients with poor-risk cytogenetics, particularly those with a complex karyotype [3]. Recent suggestions for a revised version of the IPSS also assign a higher importance to poor-risk cytogenetics [4]. Using innovative technologies such as array comparative genomic hybridization (CGH)/single nucleotide polymorphism (SNP) arrays and next-generation sequencing, a number of tumor suppressor genes were identified that are frequently inactivated by mutation, by (submicroscopic) deletion, by hypermethylation or by acquired uniparental disomy [5]. Notably, many of these genes, e.g. TET2, ASXL1, EZH2 and RUNX1, are involved in epigenetic modification of DNA and histones. These findings will not only increase our understanding of the pathogenesis of MDS, but may have clinical implications, since treatment with the DNA methyltransferase inhibitor 5-azacytidine increases overall survival in patients with higher-risk MDS compared to conventional care [6]. In this review, we

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summarize the current knowledge on cytogenetic and molecular changes in MDS, focusing on their use as diagnostic and prognostic markers to support clinical decision-making.

Cytogenetic changes

In general, genomic losses and gains are more frequent in MDS than balanced aberrations. This is in contrast to AML, where balanced translocations predominate. However, several balanced aberrations are associated with an unfavorable prognosis, e.g. inversion inv(3)(q21q26) or translocation t(3;21)(q26;q22) involving EVI1, t(1;3)(p36;q21) involving the EVI1 -related gene PRDM16 , t(6;9)(p23;q34) resulting in a fusion of DEK and CAN/NUP214 and t(11;16) (q23;p13) leading to a chimeric MLL/CREBBP gene. Providing exciting insights into the molecular pathogenesis of MDS, t(2;11)(p21;q23) leads to a strong up-regulation of the microRNA miR-125b-1 that interferes with myeloid differentiation. karyotype analysis under routine conditions, chromosome aberrations are found in about 50% of MDS and 5 and 7 chromosome aberrations are frequently seen (fig.1). In therapy-related MDS, particularly after chemotherapy with alkylating agents, chromosome aberrations are detected even more frequently, in up to 90%. In these cases, complex karyotypes are particularly common. [7,8].

Deletion 5q

Deletions (del) of the long arm of chromosome 5 (5q) occur as an isolated interstitial deletion or together with other chromosome aberrations, most frequently within a complex karyotype [7]. Isolated del(5q) is associated with a good prognosis [9]. The risk for AML transformation and overall survival depend on the presence of additional chromosome aberrations: median survival time is 58.0 months for patients with isolated del(5q)/del(5q) plus one additional aberration,and 6.8 months for patients with del(5q) plus two additional aberrations, i.e. a complex karyotype including del(5q) [10]. In the 2008 WHO classification, "MDS with isolated deletion 5q" is listed as a cytogenetically defined subtype with low blast count _ 5% and distinct clinico-morphological features such as macrocytic anemia, thrombocytosis and hypolobulated megakaryocytes [11] In 2006, List and colleagues [12] showed that the thalidomide analog lenalidomide is particularly active in MDS with del(5q). Sixty-seven percent of patients with low and 45% with intermediate-1-risk MDS with del(5q) achieve transfusion independence and complete cytogenetic remission. Patients who fail to achieve sustained erythroid or cytogenetic remission have an increased risk for clonal evolution and progression to AML. It is noteworthy that karyotyping is significantly more sensitive than fluorescence in situ hybridization (FISH) in detecting the del(5q) clone – probably due to a proliferative advantage of aberrant cells and due to the detection limit of FISH of about 10% . Thus, karyotyping should always be performed to investigate cytogenetic response and progression. Progression to AML frequently accompanies the acquisition of additional chromosome aberrations resulting in a complex karyotype. Typically, they contain del(17p) leading to a loss of TP53, del (7q) and/or trisomy 21. Even at an early phase of the disease before treatment, small clones with TP53 mutations are present in almost one-fifth of patients with low-risk MDS with del(5q) [13]. These mutations are associated with leukemic progression, sometimes years later.

Moreover, patients who later undergo leukemic progression have significantly shorter telomeres than patients who remain in complete cytogenetic remission or stable disease. During treatment with lenalidomide, telomere lengths elongate in patients with short telomeres, either by activation of telomerase or alternative telomere lengthening (ALT) or by selection of clones with long telomeres [14].There is increasing evidence that the stem cell pool from which the del(5q) clone originates may be more heterogeneous than assumed so far. In patients undergoing clonal evolution later, rare CD34+, CD38 -/low, CD90+ stem cells are distinctly resistant to lenalidomide. While the more mature progenitors with del(5q) are eradicated or largely reduced, substantial proportions of quiescent stem cells remain during treatment, even during complete cytogenetic remission. Deletions of 5q in the context of complex karyotypes are usually terminal deletions. NPM1 located in the terminal region 5q35 was suggested to be involved in the chromosomal instability leading to complex karyotypes, since Npm -deficient mice show an increased genetic instability [15].Clearly, further work is needed to fully understand diseasemechanisms in MDS with del(5q).

Monosomy 7 and deletions of 7q

Monosomy 7 is the most frequent chromosome aberration of MDS in childhood [16]. It is usually the only cytogenetically visible chromosome aberration. Previously, as in the IPSS, monosomy 7 was considered to be a poor-prognostic marker [2]. However, since the European Working Group for Myelodysplastic Syndromes (EWOG-MDS) study group decided to perform early stem cell transplant as soon as a clone with monosomy 7 was identified, prognosis of children with MDS and monosomy 7 did not differ from that of children with MDS and a normal karyotype [17]. A recent retrospective evaluation of the prognostic significance of cytogenetic findings in adult MDS confirmed that isolated monosomy 7 or monosomy 7 plus one additional aberration is associated with a median survival of 14.0 months and thus with an intermediate risk [18]. Monosomy 7 is also the most frequent chromosome aberration observed during progression to MDS and AML in patients with different bone marrow failure syndromes and DNA repair deficiencies such as congenital neutropenia, dyskeratosis congenita, Fanconi anemia and Bloom syndrome. Thus, there are underlying mutations causing these constitutional disorders that also predispose to MDS and AML. In these cases, monosomy 7 seems to be a secondary aberration driving the development of MDS and the leukemic transformation. Moreover, monosomy 7 is the most frequent chromosome aberration in juvenile myelomonocytic leukemia, a disease initiated by an activated RAS pathway, either through activating mutations of K-RAS, N-RAS, PTPN11 or CBL, or through inactivating mutations of NF-1. Again, monosomy 7 seems to be involved as a secondary aberration in leukemogenesis [19].Monosomy 7 is also recurrently seen in MDS and AML with inversion inv(3)(q21q26), leading to a rearrangement of EVI1. The retroviral vectors had integrated into the EVI1 locus.. Using high-resolution array-CGH, no recurrent genomic gains or losses were found in children with MDS and monosomy 7 [20]. Notably, patients with an isolated monosomy 7 showed significantly longer telomeres than patients with a normal karyotype in many chromosome arms, among them 7p and 7q . Deletions of 7q occur as recurrent chromosome aberrations in MDS, with a similar median survival time as for monosomy 7 if present as a sole aberration or in a noncomplex karyotype (19 months and 14 months, respectively), and are also typical aberrations of complex karyotypes [18]. Therefore, the critical genes are assumed to lie in 7q21-22 [21].Molecular mapping of a commonly deleted segment within chromosome band 7q22 in patients with AML has led to the identification of MLL5, a SET-domain containing MLL-homolog.

Trisomy 8

Trisomy 8 is the most frequent trisomy in MDS and AML [7]. Nevertheless, trisomy 8 is not listed under recurring chromosomal abnormalities considered as presumptive evidence of MDS, since +8 like – Y and del(20q) occurs in some patients with aplastic anemia or other cytopenic syndromes who have a good response to immunosuppressive therapy and/or who show no morphologic evidence of MDS with prolonged follow-up [22]. There are several reports of trisomy 8 in Philadelphia-negative clones in patients with chronic myeloid leukemia treated with imatinib mesylate. Yet, most patients did not show any signs of myelodysplasia over the years [23].Clones with trisomy 8 may already be present from birth due to constitutional trisomy 8 mosaic (cT8M). Maserati and co-workers postulated that trisomy 8 in myeloid malignancies is of constitutional origin in up to 15 – 20% of cases [24]. Recently it has been observed that, 27 patients with cT8M, 21 of whom developed hematologic neoplasms, e.g. juvenile myelomonocytic leukemia (JMML), myeloproliferative disorders, MDS and AML. It is also observed that a nearly identical gain in two patients with cT8M and JMML, dramatically reducing the critical region to 8p11.21q11.21, a region containing 31 protein coding genes and two miRNAs [25]. Clonal heterogeneity is a characteristic cytogenetic feature of MDS. As a single cell abnormality or independent clone, trisomy 8 may “come and go”. Clearly, the prognosis differs significantly if trisomy 8 occurs as an isolated abnormality, if it is present as a secondary aberration – then the prognosis depends mostly on the primary lesion – or if it is part of a complex karyotype. Usually, clonal evolution is predictive of disease progression. In line with this, patients with MDS with del(5q) and + 8 as independent clone had a significantly longer time to AML progression than those with clonal evolution and + 8 developing within the del(5q) clone [26]. Global gene expression analyses demonstrated that most, but not all genes located

on chromosome 8 are up-regulated in AML with trisomy 8. Thus, a gene-dosage effect may be responsible for the leukemic effect of trisomy 8. However, other mechanisms, e.g. doubling of a mutated, co-dominant oncogene, could also be possible. So far, these genes on chromosome 8 have not been identified.

Loss of Y chromosome

In a patient, it is diffi cult to decide whether – Y is related to the development of MDS, since – Y is also found in healthy older men [46]. As with trisomy 8, Philadelphia-negative clones with – Y are repeatedly reported in patients with chronic myeloid leukemia (CML) treated with imatinib mesylate. Pragmatically, dominant – Y clones can be considered to be possibly related to MDS. It remains unclear why – Y is so frequent in men with MDS, whereas – X is only very rarely found in women with MDS.

Complex and monosomal karyotype

In all risk classification systems, a complex karyotype is associated with a poor prognosis. Yet, the definition of a complex karyotype differs. In most instances, a complex karyotype is defined as three or more chromosome aberrations [1]. Patients with complex karyotypes containing more than three abnormalities seem to have an even worse prognosis than those with three abnormalities [11]. In 2008, Breems et al. [28] introduced the new definition of a monosomal karyotype, i.e. either the presence of at least two autosomal monosomies or the presence of one autosomal monosomy and at least one structural abnormality, as a poor-prognostic factor in adult AML. There is only limited information as to the prognostic significance of a monosomal karyotype in MDS [29]. In a series of 421 Argentinian patients, a karyotype with autosomal monosomies was associated with a median survival of 16 months, comparable to the median survival of patients with other poor-risk cytogenetics [30]. In another study on 127 patients with a complex karyotype, those who also had a monosomal karyotype had a worse prognosis than patients with a complex karyotype without monosomies. However, there was no difference in survival among patients with monosomal karyotype further stratified by the presence or absence of monosomy 7 and/or monosomy 5 [31]. This is in contrast to Schanz et al. [4], who described a hazard ratio for overall survival of 2.4 for complex karyotypes without involvement of chromosome 5 and/or 7 and a hazard ratio of 4.8 for complex karyotypes including chromosome 5 and/or 7 aberrations. Surprisingly, complex karyotypes not involving chromosomes 5 and/or 7 were associated with an intermediate risk of AML transformation in the group of patients treated with supportive care. Thus, complex karyotypes seem to constitute different prognostic subgroups.G öhring et al ., investigating children with MDS treated homogeneously with early stem cell transplant, also found that there is a group of patients with at least three chromosome aberrations (meeting the traditional definition of a complex karyotype) who have a surprisingly favorable outcome. In contrast, patients with a "structurally complex karyotype" defined as more than or equal to three chromosome aberrations, including at least one structural aberration, had a very short survival probability of only 14% [32]. Notably, the presence of a structurally complex karyotype was a better predictor of a very unfavorable prognosis in children with MDS than the presence of a monosomal karyotype. Complex karyotypes often contain a high number of chromosome aberrations that cannot be resolved based on karyotyping. What appears, for example, as monosomy 5, turns out to be rearrangements leading to deletions of various parts of chromosome 5, if molecular cytogenetic techniques such as FISH or multi-color FISH are used (Fig. 2). It is unclear whether monosomy 5 really exists in MDS and AML [33].However, this makes it difficult to be sure that a certain karyotype indeed is monosomal. In contrast, structural aberrations can easily and unequivocally be recognized. Ongoing investigations will prove whether a structurally complex karyotype allows a better risk stratification than monosomal karyotype also in adult MDS.

Molecular changes

MDS was believed to carry no specific molecular changes. RAS mutations were found not only in MDS, but also in other myeloid neoplasms. Moreover, they were not associated with a typical morphology or clinical course. This view changed rapidly when – using an elegant strategy based on SNP array analysis – TET2 mutations were identified in a substantial proportion of patients with MDS [34].Soon afterwards, three other important genes, CBL, ASXL1 and EZH2 , were identified to be recurrently

involved in MDS. Even more important, some of these genes code for enzymes involved in DNA or histone methylation. Mutation analyses of these genes will soon be part of the diagnostic process of MDS, since mutations of TP53, EZH2, ETV6/TEL, RUNX1 and ASXL1 are independent predictors of decreased overall survival, demonstrating that the detection of mutations in these genes may aid risk stratification (Table 2) [35].

TET2

Using high-resolution SNP arrays, large regions of uniparental disomy were found in 4q [34]. Uniparental disomy (UPD) means that two identical maternal or two identical paternal alleles are present. Another term used for UPD is copy-number neutral loss of heterozygosity. One can assume that the respective region contains a tumor suppressor or oncogene with homozygous (bi-allelic) mutation, because –after mitotic recombination leading to UPD – the wild-type allele is lost and the mutated allele is duplicated [36]. In the case of 4q, the critical region could be further narrowed down to small homozygous deletions. Indeed, the TET2 gene located within this region showed homozygous mutations or bi-allelic inactivation, demonstrating that it is a newly identified tumor suppressor gene in MDS. This elegant strategy paved the way for the identification of other tumor suppressor genes in commonly deleted regions.TET2 mutations were encountered in different lineages including CD34 + progenitor cells. Thus, they seem to target stem cells and to occur early in MDS development [6]. Tet proteins catalyze the conversion of 5-methylcytosine of DNA to 5-hydroxy-methylcytosine and have an important function in stem cell maintenance [37]. Notably, two other recently identified genes recurrently mutated in AML, IDH1 and IDH2 may impair its function [38]. Both mutations induce a similar genome-wide DNA hypermethylation pattern currently suspected to install a pre-leukemic state, and may also influence histone methylation .TET2 mutations occur in 14 – 26% of WHO-defined patients with MDS [34,39]. Mutated patients most often have a normal karyotype, and no mutations have been identified in patients with isolateddel(5q) .Approximately two thirds of mutated patients have a low or intermediate-1 IPSS risk score.The Groupe Francophone des My é lodysplasies reported that TET2 mutations are associated with a favorable overall survival, with a 5-year overall survival of 76.9% vs. 18.3% of mutated patients compared to wild-type, while only 10.7% of patients with MDS progressed to AML at 3 years compared to 36.3% of patients with wild-type TET2 . This effect was also seen considering only patients with lower-risk MDS (IPSS low and intermediate-1) or higher-risk MDS (IPSS intermediate-2 and high). However, TET2 mutations had no prognostic effect on overall survival in three other studies investigating patients with MDS, including one large series of 320 patients with MDS investigating TET2 mutations by next-generation sequencing [39]. The utility of the TET2 mutation as a predictive marker for treatment response to azacytidine has been suggested recently by Itzykson et al..[40] Sixty-three patients with higher-risk MDS and 23 patients with AML with 20 – 30% blasts were treated with a 7-day schedule of azacytidine every 4 weeks. Fifteen percent of patients had mutated TET2 (excluding missense mutations). Whereas overall survival (OS) was similar between mutated and wild type patients (median OS 17.5 vs. 15.3 months, respectively), the overall response rate including complete remission (CR), partial remission and marrow CR [41].was significantly larger in mutated versus wild-type patients (69 vs. 31%, p = 0.01) [42]. The fact that TET2 -mutated patients more often had good- or intermediate-risk cytogenetics, had a longer interval from diagnosis to treatment and received a higher median number of treatment cycles complicates the interpretation of these results. Also, treatment response to azacytidine in one third of patients with wild-type TET2 is clinically meaningful, and thus, additional studies are required to determine value of TET2 as a predictive marker or to identify markers with better discriminative power.

ASXL1

ASXL1 is a tumor suppressor gene recently identified using SNP array analyses and sequencing, originally identified with a frequency of 6 – 11% in patients with MDS [43]. We found ASXL1 mutations in about 20% of patients with MDS, while two-thirds of the mutations were frameshift and one-third were missense mutations [44]. ASXL1 mutations are correlated with an intermediate-risk karyotype, but not with other clinical parameters [43]. The presence of frameshift mutations in ASXL1 was associated with a shorter overall survival. ASXL1 frameshift mutations predicted a higher risk and shorter time for

progression to AML. In multivariate analysis, when considering karyotype, transfusion dependence and IDH1 mutation status, ASXL1 frameshift mutations remained an independent prognostic marker for overall survival and time to AML progression [26].In an independent study of 439 patients, ASXL1 mutations were confirmed to independently predict shorter overall survival (hazard ratio [HR] 1.38, 95% confidence interval [CI] 1.00 – 1.89, p = 0.049) [35]. These results suggest that ASXL1 mutations are frequent molecular aberrations in MDS that predict an adverse prognosis. Screening of patients for ASXL1 mutations might be useful for clinical risk stratification and treatment decisions in the future. Like TET2, ASXL1 is involved in epigenetic regulation. ASXL1 belongs to the enhancer of trithorax and polycomb (ETP) genes that can both activate or repress HOX genes. It contains several nuclear receptor binding motifs and a carboxy-terminal plant homeodomain (PHD) that is predicted to be truncated by most ASXL1 mutations found in patients [45]. ASXL1 can act as a transcriptional activator or co-repressor of retinoic acid receptor (RAR) activity depending on cellular context .Loss-of-function studies of ASXL1 did not reveal a propensity for transformation to myeloproliferation or leukemia [45]. It significantly represses retinoic acid receptor- or retinoid X receptor-dependent transcriptional activation via direct interaction with histone H3 demethylase LSD1, presumably leading to a change in histone H3K4 methylation.

EZH2

Using the same strategy as for the identifi cation of TET2 and ASXL1, the EZH2 gene located in 7q36.1 was identified. EZH2 mutations were reported with a frequency of 6% in patients with MDS [35,46]. EZH2 is involved in epigenetic control and codes for the catalytic subunit of the polycomb repressive complex 2, a histone methyltransferase that transfers methyl groups to histone H3 lysine 27 (K27). As expected, homozygous mutations were found in patients with acquired uniparental disomy of 7q. Mono-allelic and bi-allelic inactivating mutations were most frequently detected in patients with myelodysplastic/ myeloproliferative neoplasms, particularly chronic myelomonocytic leukemia, and in patients with myelofibrosis. Thus, EZH2 seems to act as a tumor suppressor gene in MDS. Notably, EZH2 mutations predict shorter overall survival in patients with MDS, as shown by Nikoloski et al. and a recent multivariate analysis in a large cohort of patients with MDS (HR 2.13, 95% CI 1.36 – 3.33, p = 0.001) [35,46]. DNMT3A In MDS DNMT3A reported to be less frequent than in AML, with an incidence of 2.6 – 8%. Similarly to AML, there appears to be a mutational hotspot at position R882. Walter et al .showed that 58% of mutated and 28% of wild-type patients progressed to AML and that DNMT3A mutation predicted a shorter overall survival [47].First insights into the biological consequences of DNMT3A mutations point to increased expression of HOXB cluster genes due to decreased promoter methylation of HOXB2 .

IDH1/IDH2

By sequencing a whole AML genome, recurring mutations were found in codon 132 of the gene for isocitrate dehydrogenase 1 (IDH1) [48]. The protein encoded by this gene is found in the cytoplasm and peroxisomes. It is a nicotinamide adenine dinucleotide phosphate [NADP (+)]-dependent isocitrate dehydrogenase and has a signifi cant role in cytoplasmic NADPH production. Mutations were soon afterwards detected in the homologous protein IDH2, which locates to the mitochondria [49]. Mutated IDH proteins function as neomorphic enzymes that catalyze the α -ketoglutarate to 2-hydroxyglutarate conversion (2-HG). 2-HG is believed to be an oncometabolite that blocks enzymatic function of many of its substrates, among others the TET family of proteins [38]. Patients with IDH-mutated AML have increased DNA methylation levels as do TET2 -mutated patients, and thus it is believed that 2-HG blocks the DNA-hypomethylating function of TET2, resulting in increased DNA methylation levels [38]. IDH1 mutations occur in 3.1 – 7.4%, and IDH2 mutations in 0 – 4.6% of patients with MDS. They are often found in normal karyotype MDS and in some patients with del(5q) [9,50]. As in patients with AML, IDH mutations in patients with MDS are associated with higher platelet counts [51]. Co-occurrence of IDH mutations with mutations in TP53, RUNX1 and JAK2 have been reported [52]. IDH1 mutations were associated with a higher rate and shorter time to AML progression and reduced overall survival [50]. In a different study, IDH1 mutations did not predict overall survival, but IDH2 mutations were associated with shorter overall survival in univariate but not multivariate analysis [35]. In patients with del(5q), a shorter leukemia-

free but not overall survival was also reported for patients with IDH1 or IDH2 mutations [9].

RUNX1

RUNX1 is involved in promiscuous translocations, such as t(12;21) in acute lymphoblastic leukemia in childhood and t(8;21) in acute myeloid leukemia, and is also targeted by point mutations and deletions [9]. RUNX1 plays a central role in early myeloid differentiation [53]. All leukemic alterations lead to an inactivation of RUNX1, by disrupting the protein complex binding to transcriptional start sites of myeloid transcription factors and initiating myeloid differentiation. Both mutations in the N-terminal Runt-homology domain and mutations in the C-terminal region induce myelodysplasia that later progresses to MDS/refractory anemia with excess blasts (RAEB) and AML in a murine transplant model [54. RUNX1 mutations collaborate with EVI1, similar to t(3;21) fusing EVI1 on chromosome 3 and RUNX1 on chromosome 21. Germ line mutations of RUNX1 predispose to MDS/AML. Some patients present with thrombocytopenia or functional defects of the platelets, which lead to the familial platelet disorder with a propensity to develop myeloid malignancies (FPDMM) [55]. The risk to develop MDS/AML depends on the acquisition of additional genetic aberrations [56], but may be up to 60%. RUNX1 mutations are found in 12 – 15.7% of patients with MDS and up to 27.7% of patients with secondary AML following MDS [58]. Moreover, RUNX1 mutations are associated with higher-risk MDS, lower platelet counts, RAS mutations and - 7/del (7q) [57], and an association with ASXL1 has been reported in patients with AML [90]. RUNX1 mutations predict a shorter time and higher incidence of MDS to AML progression, and shorter overall survival in patients with MDS compared to wild-type patients [58], now being confirmed by a recent multivariate analysis in a large cohort of patients with MDS (HR 1.47, 95% CI 1.01 – 2.15, p = 0.047) [35].

RAS

RAS encodes a G-protein involved in several pathways of signal transduction which control cell growth and differentiation. It is mutated in 20 – 30% of all tumor entities, underlining its important role for the control of cell growth. In MDS, NRAS mutations occur with a frequency of 5–15%, while KRAS mutations are very rare in MDS [59]. NRAS mutations have been associated with a negative prognostic impact on survival and disease progression [60]. However, in multivariate analysis in a large cohort of patients with MDS, NRAS mutations were not independently associated with overall survival [35].

TP53

Horiike et al. Suggested that the TP53 mutation/loss in risk assessment of MDS [61]. TP53 mutations and loss of 17p including the TP53 locus occur most frequently in therapy-related MDS (t-MDS) after treatment with alkylating agents and within complex karyotypes containing aberrations of chromosomes 5 and 7, a cytogenetic subtype with dismal prognosis [62]. TP53 mutations may have a pivotal role in the generation of amplifications and Centromeric breakpoints, leading to genomic instability. New data generated by using sensitive deep-sequencing technology indicate that TP53 mutations play an important role in disease progression in patients with low-risk MDS with del(5q) [13]. In this study, 55 patients with low-risk MDS (IPSS low and intermediate-1) with del(5q) were analyzed for TP53 mutations pre- and post progression. Patients with even a small clone of mutated TP53 had a higher rate of leukemic evolution and were less likely to respond to lenalidomide. Thus, inactivation of TP53 seems to trigger progression of MDS. In contrast, during early phases of the disease, hyper activation of TP53 as, for example, by haploinsufficiency for RPS14 induces an MDS phenotype [16].Interestingly, not only in low-risk but also in high-risk MDS, TP53 mutations appear to be a negative prognostic marker. In a recent multivariate analysis by Bejar et al., TP53 mutations were associated with a very poor overall survival (HR 2.48, 95% CI 1.60 – 3.84, p = 0.001) [35].

Rare mutations in MDS

A number of mutations occur in MDS with a very low frequency. Some of these, such as NPM1 and CEBPA, are much more frequent in AML, suggesting that some mutated genes are specific for AML development, whereas others impact both pathways that play a role in AML and MDS pathogenesis. ETV6/TEL is involved in numerous translocations, mostly in AML and in RAEB [9]. ETV6/PDGRA and ETV/PDGFRB translocations have been described in chronic myelomonocytic leukemia (CMML) and in chronic eosinophilic leukemia, but now

constitute their own entity in the updated WHO classification. Moreover, the ETV6/TEL locus is frequently deleted. Interstitial 12p deletions usually encompass ETV6/TEL and CDKN1B/KIP/P27 [63]. 12p deletions were assumed to be associated with secondary or therapy-related MDS/AML, and to confer a poor prognosis. Yet, in a recent retrospective evaluation of 2124 patients with MDS, Haase et al. [18] reported that patients with an isolated or non-complex 12p deletion have a median survival of 108 months and a very favorable prognosis. In contrast, in a recent multivariate analysis in a large cohort of patients with MDS, 2.7% of 439 patients with MDS had mutated ETV6, which independently predicted decreased survival (HR 2.04, 95% CI 1.08 – 3.86, p = 0.03) [35]. First described in a case of acute myeloid leukemia, mutations of the proto-oncogene CBL result in defective ubiquitination and degradation of tyrosine kinases and in enhanced signaling, e.g. of FLT3 and c-KIT. Soon afterwards, CBL mutations were identified in CMML, in cases diagnosed as MDS/myeloproliferative disease (MPD)-unclassifiable and in aggressive myeloproliferative neoplasms with uniparental disomy of 11q, the locus of CBL [64]. Most variants were missense substitutions in the RING or linker domains that abrogated CBL E3 ubiquitin ligase activity and conferred a proliferative advantage to the cells. This is supported by findings in the NUP98 – HOXD13 transgenic mouse model. NUP98 – HOXD13 induces a differentiation defect [65]. Collaborating mutations in RAS mutations and one mutation in CBL enhance proliferation and trigger the progression into AML. Serial studies in patients with MDS also demonstrated the acquisition of CBL mutations and clonal selection of

CBL -mutated clones during progression into AML [66]. Patients with CBL mutations have a poor prognosis, with a median survival of 5 months. However, in a recent multivariate analysis, CBL mutations were not an independent prognostic factor for overall survival [35]. Still, CBL mutations were associated with an increased blast count. JMML, an aggressive myeloid neoplasm in young children, shares similarities with chronic myelomonocytic leukemia in being categorized as myeloproliferative neoplasm (MPN)/MDS in the WHO classifi cation. In JMML, CBL mutations are mutually exclusive with RAS/PTPN11, indicating that CBL may play a role in deregulating the Ras pathway. Surprisingly, children with variable dysmorphic features carry a heterozygous germline CBL mutation [67]. In leukemia cells, homozygous mutations are present due to mitotic recombination and UPD of 11q. Thus, germline CBL mutations predispose to JMML, although additional aberrations may be necessary for overt leukemia. Germline mutations in CEBPA also cause familial leukemia, probably at an earlier age than sporadic leukemia. There is a report on donor cell leukemia in a patient and his donor sister, who both had the same germline mutation in CEBPA [68]. Leukemia development requires additional genetic changes, mostly a mutation of the second allele and in some instances chromosome aberrations such as -7. To date, mono-allelic and bi-allelic mutations of CEBPA have been described in cytogenetically normal AML. In MDS, CEBPA mutations seem to be rare and may be involved in the progression to AML. NPM1 and WT1 are frequently mutated genes in AML but rarely occur in MDS. Similarly, the JAK2-V617F point mutations are very common in chronic myeloproliferative neoplasms but again occur in MDS with a very low frequency, except in the subgroup of patients with refractory anemia with ring sideroblasts (RARS) with marked thrombocytosis, where 50% of patients are mutated [68].

NIIH experience

The cytogenetics laboratory at our Institute actively involved in conducting studies on MDS. Cytogenetics including FISH studies were carried out in more than 300 MDS patients. Our studies showed that the Indian MDS patients were different from western literature as our majority of patients were young or below 45 years age group [69]. Cytogenetic studies from our Institute revealed 54% chromosomal aberrations including rare chromosomal aberrations [69-71]. Some group of patients found to be occupationally exposed, particularly pesticides and these patients showed significantly high frequency of chromosomal breakages [72]. We have also carried out study to detect DNA copy number changes using comparative genomic hybridization in karyotypically normal MDS patients. Our results suggested that chromosome 1 and 7 are frequently involved in DNA losses and gains [73]. However, detailed molecular studies are essential to know the disease pathology. We have also initiated molecular studies in MDS to look gene mutations in TET2, ASXL1, IDH1, IDH2, Jak2, p53 genes to understand molecular pathology of the disease.

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Table 1: Cytogenetic risk groups according to IPSS and WPSS (2-4)

Cytogenetic risk group	Chromosome aberration	IPSS risk score	WPSS risk score
Good	Normal karyotype,-Y,isolated del(5q), isolated del (20q)	0	0
Intermediate	All other aberrations	0.5	1
Poor	-7, del(7q) and other abnormalities of chromosome 7, complex karyotype(<3 aberrations)	1.0 (2.0) (4)	2

IPSS: International Prognostic Scoring System; WPSS: World Health Organization classification- based Prognostic Scoring System

Table 2: Molecular abnormalities in recurrent chromosome aberrations of MDS

Locus	Gene	Mutation	Affected pathways
3q26	EVI1	Translocation/ Inversion	Signal translocation
4q24	TET2	UPD, Mutation	Epigenetic modification
5q31	RPS14 SPARC EGR1 CTNNA1 (α-catenin)	Haploinsufficiency Haploinsufficiency Haploinsufficiency Promoter Hypermethylation?	Ribosomal protein Signal transduction Signal transduction Cell-cell contact
7q22	MLL5	Methylation	Epigenetic modification
7q36	EZH2	UPD,deletion,mutation	Epigenetic modification
9p24	JAK2	V617F mutation	Signal transduction
11p15	NUP98	Translocation	Signal transduction
11q23	MLL CBL	Translocation UPD	Epigenetic modification Signal modification
12p13	TEL/ETV6	Translocation/deletion	Signal transduction
17p13	TP53	Deletion, Mutation	Signal transduction
20p12	PI-PLCβ1	Deletion	Signal transduction
20q11	ASXL1	Deletion,Mutation	Epigenetic modification
21q22	AML1/RUNX1	Translocation/mutation	Myeloid differentiation

MDS, myelodysplastic syndromes; UPD,uniparental disomy.

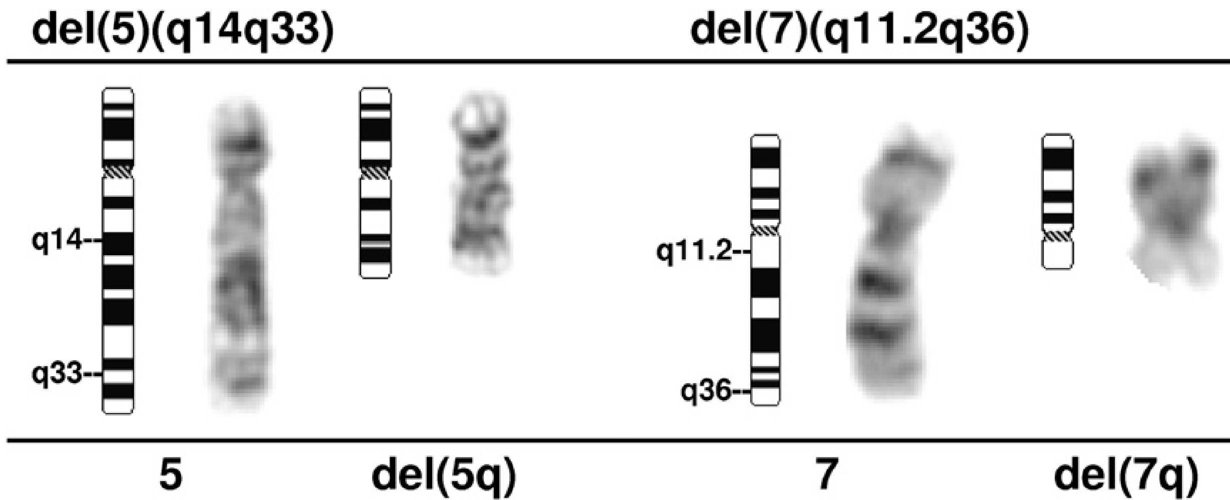

Fig.1. Deletions of 5q and 7q in myeloid neoplasms. In this del(5q), breakpoints occur in q14 and q33 resulting in interstitial loss of the intervening chromosomal material. In this del(7q), breakpoints occur in q11.2 and q36. In both cases, the critical commonly deleted segments are lost. Normal chromosome 5 and 7 homologs are shown for comparison.

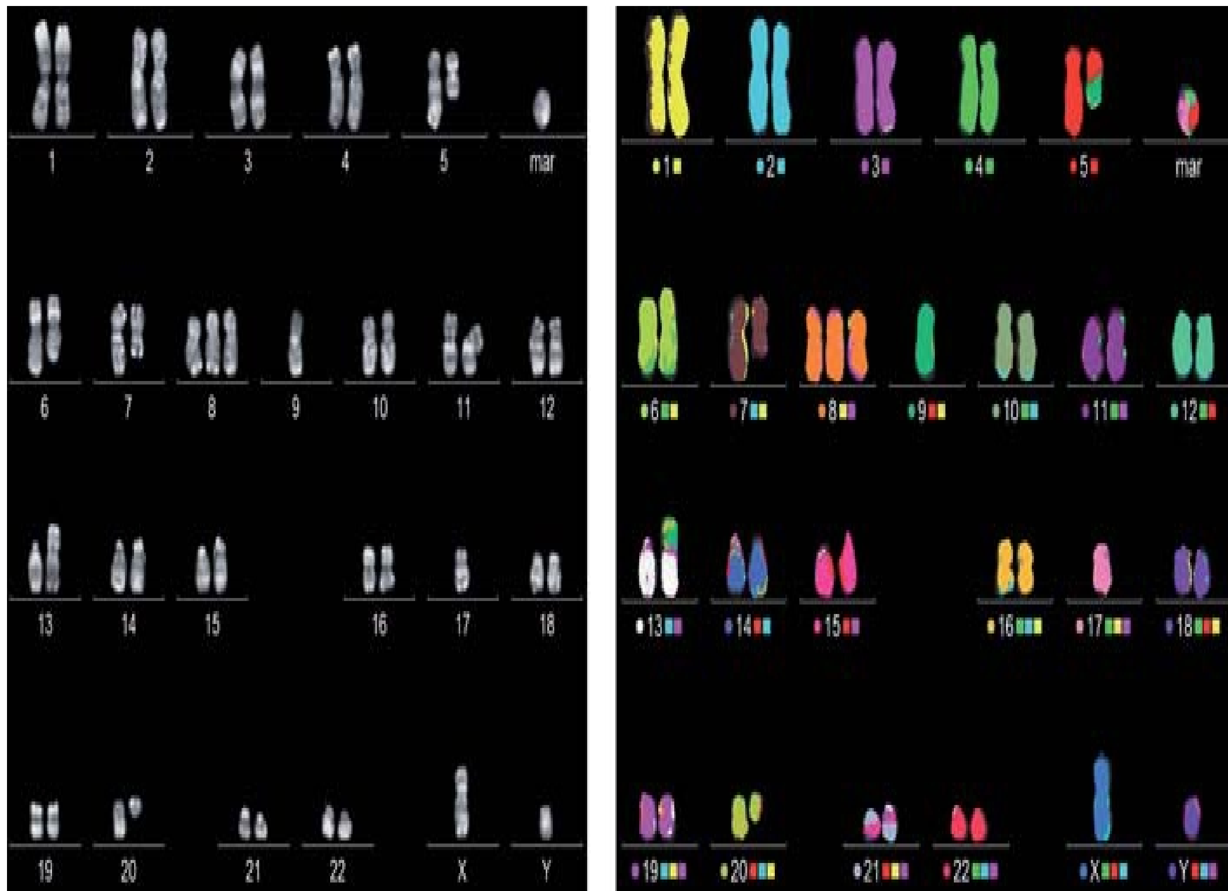

Figure 2. Karyogram after fluorescence R banding and after multicolor fluorescence in situ hybridization of a patient with MDS and a complex karyotype

A NOVEL ELISA FOR DIAGNOSIS OF GLANZMANN'S THROMBASTHENIA AND THE HETEROZYGOTE CARRIERS

Vivian Lobo

Glanzmann's thrombasthenia (GT) is a rare autosomal recessive bleeding disorder, caused by a quantitative or qualitative defect of the platelet glycoprotein GP IIb–IIIa receptors [1]. It is caused by mutations in the genes encoding the two platelet glycoproteins, i.e., ITGA2B and ITGB3 [2]. Both GPIIb and IIIa are the products of separate genes and they form a heterodimer complex after synthesis, which undergo final processing and transport to the platelet membrane. The clinical manifestations include lifelong bleeding with easy bruisability, epistaxis, menorrhagia, and gastrointestinal bleeding [3]. GT occurs in high frequency in certain ethnic populations with an increased practice of consanguineous marriages, such as Iraqi Jews, Indians, Palestinian and Jordanian Arabs, and French Gypsies [4–6]. Patients with less than 5% of normal αIIbβ3 are classified as type 1 and those who show the expression between 5% and 20% as type 2. Type 3 variants usually have dysfunctional receptors with normal αIIbβ3 levels.

The laboratory diagnosis of GT depends on a series of investigations involving platelet aggregation, clot retraction tests, flow cytometry, platelet procoagulant activity and molecular biology. Direct gene analysis is the most accurate method of carrier detection. However, these tests are not widely available and are not specific for GT and also they are expensive and require expertise. Platelet aggregation as a single test can be diagnostic if the results are repeatedly similar and consistent with clinical presentation [6]. Western blotting using platelet lysate has also been employed for diagnosis of GT however it has its own limitations.

An ELISA for detection of index cases and heterozygotes of GT has been established which is cost effective, sensitive, specific, and easy to perform, detect and conclude. The technique involves the use of anti CD36 to coat the platelets in microtiter plates which would then be revealed using echistatin conjugated with alkaline phosphatase. The methodology in detail is as follows:

Methodology:

Preparation of platelet suspension: Venous blood was collected in trisodium citrate vacutainers and blood was centrifuged at 700 rpm for 15 min at room temperature (RT) within 30 min of collection. The platelet-rich plasma (PRP) was treated with prostaglandin (PGE1) (Sigma Aldrich, MO, USA) to a concentration of 1 ng/ml of the PRP and was spun at 450 g for 15 min at RT. One millilitre of 1% ammonium oxalate was added to the residue to lysethe red cells. After centrifugation at 450 g for 15 min at RT, 1 ml of Ringer's citrate dextrose (RCD pH 5.4) buffer was added to the pellet followed by washing with PBS-Tween 20three times. The final suspension of platelets was made in RCD buffer and the platelet count was adjusted to 70,000/µl, 35,000/µl, and 17,500/µl using the same buffer.

ELISA protocol: The wells of the flat bottomed F16 NUNC plates (Nunc, Roskilde, Denmark) were precoated with100 µl of 1:100 dilution of anti-CD36 (BD Pharmingen,San Diego, CA, USA) and incubated overnight at 4°C. Allthe remaining steps were carried out at RT. The plateletswere prechecked for activation by using CD62 antibody(BD Pharmingen, San Diego, CA, USA). The platelet suspensionafter adjusting the count was added to these platesand incubated for 2 h. The excess of platelets was removedby washing the wells three times with wash buffer. To thiswas added 100 µl of echistatin conjugated with alkalinephosphatase (diluted to 1:100 with RCD buffer) and wasincubated for an hour at RT. After washing three times,100µl of substrate, i.e., para nitrophenyl phosphate (2 mg/ml in 0.1 M sodium carbonate buffer pH 9.8) (SigmaAldrich, MO, USA) was added and incubated in dark for1 h. The reaction was stopped by adding 50 µl of

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3N NaOH and the A405 was read in a spectrophotometer.

All the tests were performed in triplicates on the same day and ELISA was repeated on the precoated plates after 7 days to assess the interassay variation. Both inter and intraassay variation has been found to be below 5% and within acceptable limits.

Platelet ELISA carried out by the above method was sensitive enough to detect platelets to a count of approximately 350–400 cells/µl. The ELISA was also sensitive to detect heterozygote carriers of GT. Platelets from other platelet function defects like Bernard Soulier syndrome (BSS) showed normal optical density (OD) values in the present ELISA indicating the specificity of this platelet ELISA.

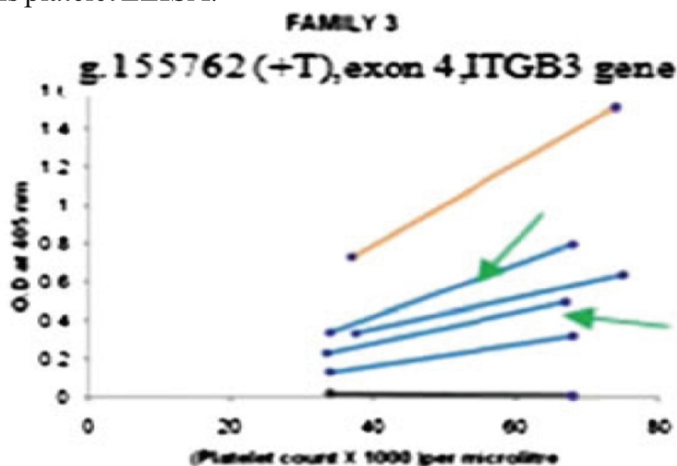

Fig.1 Orange line in the graphs indicatesnormal controls. Blue linein the graphs indicates GT carriers. Black line in the graphs indicatesGT patients. Family 3 shows the new heterozygotes (indicated by twogreen arrows in the graph) detected and subsequently confirmed bymutation, i.e., 155762 (+T) in exon 4 of ITGB3 gene

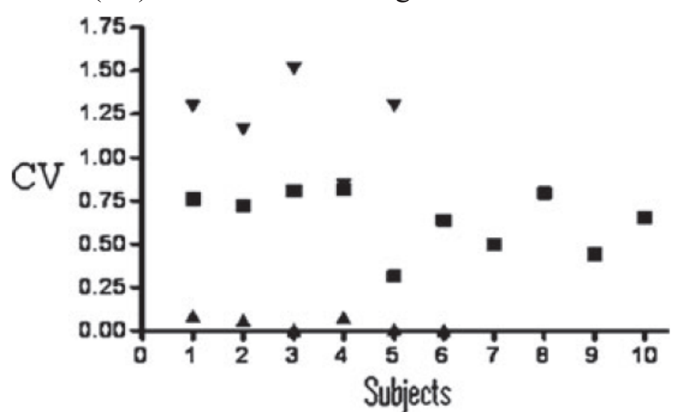

Fig.2 ANOVA biostatistic carried out for the OD values obtained from the family studies (P value<0.0001)

In conclusion, the ELISA described here is a simple screening approach to reach a diagnosis of congenital GT in parts of the world where access to advanced techniques such as flow cytometry or platelet aggregometry may be unavailable. This relatively simple test performed with excellent operating characteristics can also be useful as a screening technique for the detection of carriers in communities or parts of the world wherein GT is common.

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NIIH Library and Information Centre has been the key source for the research activity of the Institute. At present, it is equipped with modern amenities like WI-FI, Online databases and Consortium. It is regarded as one of the best Bio-medical & Health Science Research Libraries in Mumbai. In accordance with the objectives of the research, the library aims to develop a comprehensive collection of documents (both online and offline). Library is also doing bibliometric analysis of NIIH scientific publications.

Bibliometrics and Scientometrics

Bibliometrics and scientometrics are two closely related approaches to measuring scientific publications and science in general, respectively. In practice, much of the work that falls under this header involves various types of citation analysis, which looks at how scholars cite one another in publications. This data can show quite a bit about networks of scholars and scholarly communication, links between scholars, and the development of areas of knowledge over time.

The term bibliometrics was coined in 1969 by Alan Pritchard. He defined bibliometrics as "The application of mathematical methods to books and other media of communication."

Why are bibliometrics important?

Increasingly bibliometrics are being used as a measure of research impact or research influence. Bibliometrics analyses quantitative and qualitative data to describe publication patterns within a field of research. This information can be used to evaluate the influence/ performance of a researcher and to provide a comparison between researchers. More broadly, the results also help to determine Institute's rankings and have an impact on Institute funding.

Bibliometrics measures

The most commonly used measures to assess the impact of a particular publication or of a particular researcher are:

Impact factor: a measure of the impact of a particular journal using JCR.

Other journal-based metrics including SCImago Journal & Country Rank and Eigen factors.

h-index: a measure of your personal impact using Web of Science

Times cited: find how often your papers have been cited.

Tools used to measure bibliometrics

There are three main tools to measure bibliometric data: Web of Science, Scopus, and Google Scholar. Each has certain advantages and limitations which may influence which source or combination of sources you decide to use in your bibliometric search.

Impact factors

The impact factor of a journal is a quantitative tool for evaluating the relative importance of a journal. It is a measure of the frequency with which its published papers are cited up to two years after publication. To find out an impact factor use Journal Citation Reports. JCR is available within Web of Knowledge and is the key source of information about the impact of a journal, giving impact factors, cited half-life and immediacy index for each title. JCR covers specialties in the areas of science, technology, and the social sciences and is updated annually in two editions. The Science edition covers over 5,000 journals; the Social Sciences edition covers over 1,500 journals.

Vijay Padwal, ALIO

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K.E.M.Hospital campus, Parel, Mumbai 4000012. (India). Email: vgpadwal@gmail.com

Impact Factor = Number of Citations in the year/Number of Published articles in previous 2 years.

Example: Annual Review of Medicine

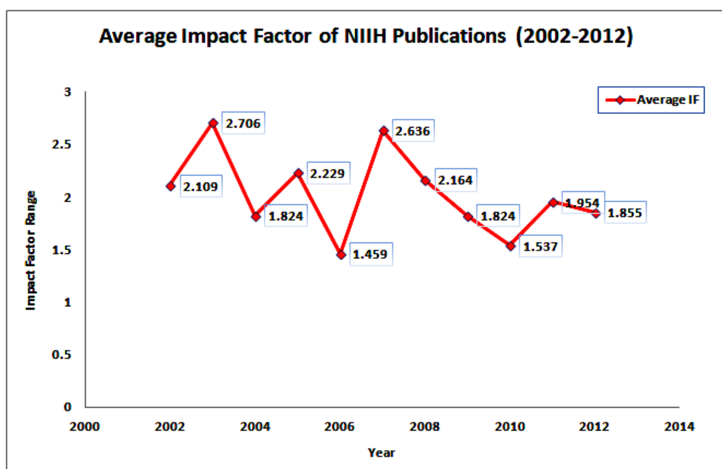
In "AR Medicine" total articles published in 2009-2010 = 36+33= 39

The total citations received by 39 articles in 2011 = 4862

Impact Factor of AR Medicine in 2011= 4862/39= 70.463 %

Average Impact factor of NIIH publications for the last 11 years i.e. from the year 2002 to 2012 has been calculated and the dotted curve graph is depicted as shown in the figure below. The last 5 years impact factor of NIIH publications is 1.866

Figure 1:



h-index

The h-index (or Hirsch index) is a relatively new, but increasingly important, method of assessing the impact of an individual's publications. The h-index uses a calculation based on the citation rates of an author's published papers.

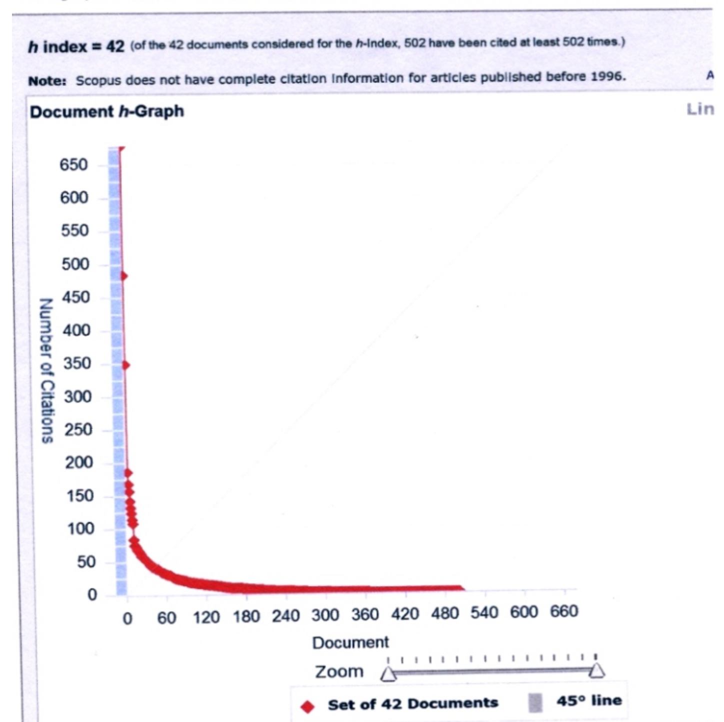
How to calculate your h-index

The Library has produced two guides on how to calculate your h-index.

Finding your h-index in Web of Science

Finding your h-index in Google Scholar

Bibliometric Analysis of NIIH Scientific Publications



Bibliometric analysis of Scientific publication of NIIH scientists were carried out for the last 11 years i.e.2002-2012. Out of 502 publications in peer reviewed journal all set of 502 documents h-graph is measured. Of the 502 considered for h index, 42 have been cited at least 42 times. The h-graph measures the impact of a set of articles and shows the number of citations per document. The publication trend is depicted below in Figure.2

Publications

Annual Report of the Institute for the year 2012-2013 was compiled and published.

Some Recent Additions (Apr 2012 – Mar 2013)

Books	-	12
Journals	-	International- 38
		National-15
E-Journals	-	28
Bound Volumes	-	500
CD-ROM/DVD	-	36

NIIH HAPPENINGS

Dr. K. Ghosh, Director

1. Attended "IHTM Haematology Master Class 2013" held at Institute for Haematology and Transfusion Medicine, Kolkata from 12th -13th January 2013 and delivered a Lecture on "Peripheral Smear Examination".
2. Attended the concluding meeting of Jai Vigyan Mission Project Phase II at CMC, Ludhiana on 16th and 17th January 2013.
3. Invited as a Guest at Nashik Homeopathic Medical College Convocation Programme held at Nashik on 24th January 2013.
4. Invited to deliver the Prafulla Bala Kiran Shasi Nag Memorial Oration at Kolkata on 28th January 2013. The topic of the Oration was "Stem Cell saga: A revaluation in the malignancy".
5. Organised the 2nd International PID Conference at Hyatt Regency, held at Mumbai from 2nd-4th February 2013.
6. Organised a training programme for the Translational Research for the Technicians and Medical Officers from 18th - 20th February 2013.
7. Attended the Scientific Advisory Group Meeting held at ICMR, New Delhi on 28th February 2013.
8. Attended a Conference on "Haematological Clinical Malignancies" held at NRS Medical College, Kolkata on 2nd - 3rd March 2013 and delivered a lecture on "Factor VIII Inhibitor and its management".
9. Attended the Tribal Health Research Forum Meeting held at NIE, Chennai on 25th March 2013.
10. Attended the Tribal Health Research Forum Meeting held at RMRC, Puducherry on 15th April 2013.
11. Invited as a Selection Committee Member to select PDF candidates at ICMR, New Delhi on 16th April 2013.
12. Invited to give a talk on "Pathogenesis of anemia in relation to malarial infections" at the 24th Congress of Parasitology at RMRC, Jabalpur on 26th and 27th April, 2013.
13. Visited Indira Gandhi Medical College and Hospital, Nagpur as a member of the ICMR Expert Team for examination of the facilities for diagnosis and treatment of sickle cell disease available and for improvement of the same on 29th April, 2013.

Hemato-Genetics

Dr R Colah, Scientist F

1. Organized a workshop on "Diagnosis and Management of Thalassemia, Sickle Cell Anemia and Hemophilia for doctors, technicians and genetic counselor from 3 districts of Maharashtra, Satara, Thane and Amravati for Maharashtra State Government from 1st to 4th January, 2013.
2. Invited to give a talk on "Laboratory Diagnosis of Thalassemia and other Hemoglobinopathies using HPLC" at the CME on "Latest Trends in Hemoglobinopathies" organized by Dept of Biochemistry, Grant Medical College on 4th January, 2013 at JJ Hospital, Mumbai.
3. Invited to participate in an Expert Working Conference on Improving the diagnosis and management of β-Thalassemia and Hb E - β-Thalassemia in India and Bangladesh from 8th -10th January, 2013 at Institute of Haematology and Transfusion Medicine, Kolkata.
4. Attended Tribal Health Research Forum meeting at ICMR, New Delhi on 12th January, 2013.
5. Invited to give a talk at the CME for Gynaecologists and Pediatricians on "Thalassemia Awareness: Role of Molecular Genetics" at CMC, Ludhiana on 16th-17th January, 2013.
6. Invited as a Moderator for the Panel Discussion to give a talk at the CME on "Laboratory Diagnosis of Hemoglobinopathies-Diagnostic Issues". at the

National CME in Hematology, Bombay Hospital, Mumbai, 23rd January, 2013.

7. Organized the Expert Group Meeting for evaluation of the RDB kits being prepared for ICMR by Imgenex India Pvt.Ltd on 31st Jan 2013 at NIIH, Mumbai.

8. Attended the Red Cross Blood Transfusion Sub-Committee meeting at Mumbai on 13th March, 2013.

9. Attended the BioRad HPLC Users Meet at Mumbai on 15th March, 2013.

10. Organized a workshop on "Molecular and Prenatal Diagnosis of Hemoglobinopathies and Screening and Diagnosis of G6PD deficiency for ICMR Institutes involved in this work under the Tribal Health Research Forum at NIIH, Mumbai on 19th and 20th March, 2013.

11. Visited Agartala Medical College, Tripura for giving training and initiation of the DBT project on "Newborn screening for red cell enzymopathies and hemoglobinopathies" from 4th – 6th April, 2013.

12. Attended the ICMR Tribal Health Research Forum meeting at Pondicherry on 15th April, 2013.

13. Invited to give a talk on "Distribution of Hemoglobinopathies in India with reference to P.falciparum malaria" at the 24th Congress of Parasitology at RMRC, Jabalpur on 26th and 27th April, 2013.

14. Visited Indira Gandhi Medical College and Hospital, Nagpur as a member of the ICMR Expert Team for examination of the facilities for diagnosis and treatment of sickle cell disease available and for improvement of the same on 29th April, 2013.

Dr M Mukherjee, Scientist D

1. Co-Organizer for the training programme on "Thalassemia, Sickle Cell Anemia and Hemophilia" for Medical officers, Technicians, Social Workers and Physiotherapists for NRHM, Maharashtra held at NIIH, Mumbai from 1st to 4th January 2013.

2. Attended "Tribal Health Research Forum Meeting" held at ICMR, New Delhi on 12th January 2013.

3. Attended "International Conference on Next Revolution in Genetics & Genomics - Applications in Health and Disease" held at New Delhi from 27th to 29th January 2013 and presented a paper entitled "The effects of UGT1A1, HMOX1 and Fcγ receptor polymorphisms on hyperbilirubinemia in sickle cell disease".

4. Attended "High Power Committee Meeting" on "Independent evaluation of performance of ICMR specialty regarding the activities to be carried over to XII plan" at ICMR, New Delhi on 28th January 2013.

5. Attended "Condemnation Committee Meeting" held at ICMR, New Delhi on 31st January 2013.

6. Treasurer for the 2nd International Conference on Primary Immunodeficiency Diseases held at Mumbai from 2nd to 4th February 2013.

7. Attended "3rd Hemoglobin Update Meets" held at Mumbai on 15th March 2013.

8. Co-Organizer for the Workshop on "New Born screening for Hemoglobinopathies and G6PD deficiency" held at NIIH, Mumbai from 19th to 20th March 2013 for the Scientists and Technical personal under the THRF programme.

9. Attended "36th Annual Conference of Mumbai hematology Group" held at Mumbai from 23rd to 24th March 2013.

10. Visited Agartala Medical College, Tripura for training and to initiate the DBT sponsored project on "Newborn screening for red cell enzymopathies and hemoglobinopathies" from 4th – 6th April, 2013.

11. Attended "Tribal Health Research Forum Meeting" held at VCRC, Pondichery on 15th April 2013.

Dr . Anita Nadkarni, Scientist D

1. Attended International conference on "Next Revolution in Genetics and Genomics, Application in Health and Disease" held at RML hospital Delhi from 27th -29th March 2013 and presented a Poster entitled "Beta globin gene analysis of borderline

HbA2 individuals: Implication for prenatal diagnosis".

2. Participated in Hemoglobinopathies Updates and 10th Bio-Rad Thalassemia user meet held at Mumbai on 15th March 2013

3. Attended 36th Annual conference of Mumbai Hematology group held at Mumbai from 23rd to 24th March 2013 .

Dr P S Kedar, Technical Officer

1. Attended "Representative of the Federation of All India ICMR Employees (FAIIE) meeting" held at ICMR Headquarter New Delhi on 25th Feb 2013.

Students

1. Dr. Khushnooma Italia attended 36th Annual conference of Mumbai Hematology group held at Mumbai from 23rd to 24th March 2013 and awarded the 2nd Prize for the Poster Presentation on a paper entitled "The Variable Clinical and Haematological Presentation of HbSD Disease".

2. Selma D'Silva attended 36th Annual conference of Mumbai Hematology group held at Mumbai from 23rd to 24th March 2013 and awarded Dr J C Patel and Dr HM Bhatia best paper award for the year 2013 by the Mumbai Hematology Group for the research paper entitled "Genetic risk factors related to unconjugated hyperbilirubinemia among the neonates in India"

3. Pooja Dabke attended 36th Annual conference of Mumbai Hematology group held at Mumbai from 23rd to 24th March 2013 and presented a paper entitled "Effect of linked and unlinked genetic modulators of beta thalassaemia on the clinical severity of the disease".

4. Dipti Upadhye attended 36th Annual conference of Mumbai Hematology group held at Mumbai from 23rd to 24th March 2013 and presented a poster entitled "Effect of alpha thalassaemia on the hematological parameters in newborns with sickle cell disease".

5. Stacy Colaco attended 36th Annual conference of Mumbai Hematology group held at Mumbai from 23rd to 24th March 2013 and presented a poster entitled "Effect of the most common Indian delta globin gene mutation -68 (C-> T) on carrier detection of beta thalassaemia".

Hemostasis and Thormbosis

Dr. S Shetty, Scientist E

1. Invited as a Panelist for the Panel Discussion on " Thrombotic Thrombocytopenic Purpura" at the 36th Annual Conference of Mumbai Hematology Group (MHG) held at Kokilaben Dhirubhai Ambani Hospital & Medical Research Institute, Mumbai from 23rd -24th March 2013.

Sharda Shanbhag, Technician C

1. Presented a poster entitled 'Heterozygote frequency of common polymorphic markers in F13A gene' in the 36th Annual Conference of Mumbai Hematology Group (MHG) held at Kokilaben Dhirubhai Ambani Hospital & Medical Research Institute, Mumbai from 23rd -24th March 2013.

Students

1. Tejasvita Gaikwad delivered a lecture on "Warfarin Induced skin necrosis" in Mumbai Hematology Group meeting held at National Institute of Immunohematology, Mumbai on 4th Jan 2013.

2. Tejasvita Gaikwad presented a poster entitled 'Factor V Leiden mutation protects from warfarin induced bleeding' at the 36th Annual Conference of Mumbai Hematology Group held at Mumbai from 23rd -24th March 2013.

3. Rucha Patil was awarded J.C Patel and Dr H.M. Bhatia Award at Mumbai Haematology Group 2013 for oral presentation entitled "Elevated procoagulant microparticles in women with recurrent pregnancy loss" (2013) in the 36th Annual Conference of Mumbai Hematology Group held at Mumbai from 23rd - 24th March 2013.

Pediatric Immunology and Leukocyte Biology

Dr Manisha Madkaikar, Scientist E

1. Invited as Coordinator for the panel discussion in 'XIXth National CME in Haematology & Haemato-Oncology' held at Bombay Hospital on 26th Jan 2013.
2. Delivered a lecture on 'Leukocyte Adhesion Deficiency Syndromes' in the '2nd International Conference on Primary Immunodeficiency Diseases' held at Mumbai from 2nd - 4th Feb 2013,.
3. Awarded BGRC Oration by Mumbai Hematology Group in the annual conference held on 23rd -24th march 2013 on a topic entitled "Diagnosis of Primary Immunodeficiency Disorders: Our journey so far".

Ms Maya Gupta, Technical Assistant

1. Awarded best poster award for a poster entitled "'Diagnosis of Primary Immunodeficiency Disorders by flowcytometry" in the 2nd International Conference on Primary Immunodeficiency Diseases' held at Mumbai from 2nd - 4th Feb 2013.

   Following papers were presented in the '2nd International Conference on Primary Immunodeficiency Diseases' held at Mumbai from 2nd - 4th Feb 2013.

   1. Dr Sushant Chavan: "Molecular pathology of Leukocyte adhesion deficiency–I in Indian patients: Identification of 6 novel mutations".
   2. Ms Snehal Mhatre: "Rapid flow cytometry based assays for NK cell function evaluation for diagnosis of Hemophagocytic Lymphohistiocytosis (HLH)".
   3. Ms Snehal Mhatre: "Spectrum of Perforin gene mutation in Familial Hemophagocytic Lymphohistiocytosis(FHL) patients in India".
   4. Ms Aparna Dalvi: "Molecular Characterization of X-Linked Hyper IgMsyndrome caused due to mutations in CD40LG".
   5. Ms Anju Mishra: "Spectrum of Primary Immunodeficiency Disorders from a tertiary referral Center in India.

Cytogenetics

Dr V Babu Rao, Scientist D

1. Nominated as Member, Institutional Ethics Committee, SRL laboratories, Mumbai on 3rdJanuary 2013.
2. Attended XIXth CME in Haematology & Haemato-Oncology held at Bombay Hospital Institute of Medical Sciences,Mumbai, from 23rd to 26th January 2013. Moderator for the Panel discussion on "Karyotyping & FISH: Basics & Applied Aspects.
3. Elected as Executive Committee, Member, Indian Society of the Human Genetics for the year 2013-2014.
4. Nominated as Chairman, Institutional Ethics Committee, Surat Raktadan Kendra & Research Centre, Surat.

Students

1. Shantashri Vaidya, attended "International conference on Molecular Pathology and 2nd Annual Conference of Molecular Pathology Association of India," held at Raipur from 9th – 10th February 2013 and presented a paper entitled "Tyrosine kinase domain mutations in imatinib resistant chronic myeloid leukemia".
2. Dolly Joshi, attended "International conference on Molecular Pathology and 2nd Annual Conference of Molecular Pathology Association of India," held at Raipur from 9th – 10th February 2013 and received best paper award, for oral presentation on a paper entitled "MicroRNA Expression Analysis in CML patients with derivative 9q deletion".
3. Shantashri Vaidya, attended Joint conference of HGM 2013 and 21st International Congress of Genetics, held at Singapore from 13th – 18th April 2013 and made a Poster presentation entitled "Tyrosine kinase domain mutations and CYP3A4/5

gene polymorphisms in imatinib resistant chronic myeloid leukemia”.

Transfusion Medicine

Dr A Gorakshakar, Scientist E

1. Invited to deliver a talk entitled “Epidemiology of Thalassemia in India” at the “Public Health Conference 2013 (Pregnancy loss, birth defects and genetic disorders in India: Epidemiology, social costs, health systems needs)” held at Pune from 11th -12th February 2013 organized by Interdisciplinary School of Health Sciences , University of Pune.

Dr Swati Kulkarni, Scientist B

1. Received ICMR International Fellowship for Senior Scientist 2012-13 and was trained for non invasive fetal RhD typing under Prof. Robert Flower, Research Programme Leader, Australian Red Cross Blood Service, Queensland, Australia from 14th - 28th Jan, 2013.

Clinical and Experimental Immunology

1. Prathamesh Surve attended 2nd Annual Conference of MPAI & International Conference on Molecular Pathology held at Raipur, from 9th -10th February, 2013 and presented a poster entitled “The Mannose Binding Lectin (MBL) gene Polymorphism: Role in clinical presentation and autoantibody production in Systemic Lupus Erythematosus (SLE)”

Transfusion Transmitted Diseases

1. Organized the HIV EQAS Panel distribution workshop for the Technicians working in various State Reference Laboratories, Mumbai from 8th – 9th January 2013.
2. Pre-assessment for NABL accreditation of NRL wing of the Department of Transfusion Transmitted Diseases, was conducted by Dr. Ranganathan Iyer (NABL Assessor) on 23rd March 2013.

Administration

1. Vijay Padwal, ALIO Attended “Representative of the Federation of All India ICMR Employees (FAIIE) meeting” held at ICMR Headquarter New Delhi on 25th Feb 2013.
2. Mr. Shankar Anbhavane, Technician C super annuated on 28th February 2013 after compilation of 40 years of service.
3. Anita Mukherjee, PS and Vijay Padwal, ALIO attended the Faculty Development Programme on Research Methodology held at Thakur Institute of Management Studies and Research, Mumbai from 5th -9th March 2013.

OBITUARY

Dr. Sharad Vishwanath Apte, MD, Ph.D., former Director of National Institute of Immunohaematology, Mumbai passed away on 2nd February 2013 at Pune after a few days of illness. Born on 7th July 1930 in Pune, he obtained his MBBS degree from B.J.Medical College, Pune University in 1955. He completed his MD in Biochemistry in the year 1963 from Osmania University. Later on he also obtained Ph.D. degree under the guidance of Dr C Gopalan in the year 1969 from Poona University. The title of his thesis was 'Studies on Iron metabolism'.

He joined as a Research worker at NIN Hyderabad and over the years rose to Senior Deputy Director General and head of Basic Medical Science Division of ICMR, New Delhi. While working at NIN he received Rockfeller Foundation fellowship during 1965-1967. He published his research work mainly on iron balance in international journals like Lancet, British J Nutrition, American J Clin. Nutrition, British J Haematology, IJMR etc.

During his tenure at ICMR he mentored 'National Talent Search Programme' which was the brain child of Dr. C. Gopalan, then Director General, ICMR. With the help of this programme several bright medical students were taken to path of medical research at National level. Many of them opted to join ICMR and today they rose to the top position in ICMR. To name a few are Dr. V.M.Katoch, Secretary Dept. of Health Research and Director General, ICMR, Dr. K Ghosh, Director, NIIH, Mumbai, Dr. S.K. Kar, Director, RMRC Bhubaneshwar. He also developed two more programmes viz Short Term Research Studentship and Research Career Development Fund for mid level faculty in medical colleges.Thus Dr. Apte was responsible to develop a solid foundation of ICMR. Dr. Apte was an accomplished medical administrator with a keen eye for the details under his guidance several advanced centres funded by ICMR flourished.

He was instrumental in acquiring 13th floor for NIIH. Later on in 1988, he took over the charge as Director. Dr. Apte was mainly responsible for furnishing and modernizing all the laboratories. He managed to get several latest equipments required for research work. This helped the scientists to initiate advanced research in various facets of Immunohaematology.

May God rest his soul in peace.

Dr. Ajit C. Gorakshakar

Scientist E