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RESEARCH ARTICLE

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Refined cytogenetic IPSS-R evaluation by the use of SNP array in a cohort of 290 MDS patients

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Abstract

Genetic testing plays a central role in myelodysplastic neoplasms (MDS) diagnosis, prognosis, and therapeutic decisions. The widely applied cytogenetic revised international prognostic scoring system (IPSS-R) was based on chromosome banding analysis (CBA). However, subsequently developed genetic methodologies, such as single nucleotide polymorphism (SNP) array, demonstrated to be a valid alternative test for MDS. SNP array is, in fact, able to detect the majority of MDS-associated cytogenetic aberrations, by providing further genomic information due to its higher resolution. In this study, 290 samples from individuals with a confirmed or suspected diagnosis of MDS were tested by both CBA and SNP array, in order to evaluate and compare their cytogenetic IPSS-R score in the largest MDS cohort reported so far. A concordant or better refined cytogenetic IPSS-R array-based score was obtained for 95% of cases (277). Therefore, this study confirms the effective applicability of SNP array toward the cytogenetic IPSS-R evaluation and consequently, toward the molecular international prognostic scoring system for MDS (IPSS-M) assessment, which ensures an improved MDS risk stratification refinement. Considering the advent of additional genetic technologies interrogating the whole genome with increased resolutions, counting cytogenetic abnormalities based on their size may result in a simplistic approach. On the contrary, assessing overall genomic complexity may provide additional crucial information. Independently of the technology used, genetic results should indeed aim at ensuring a highly refined stratification for MDS patients.

KEYWORDS

cytogenetic IPSS-R comparison, genetic testing in MDS, genomic complexity, MDS cohort, MDS patient stratification, refined score, SNP array

1 | INTRODUCTION

Myelodysplastic neoplasms (MDS) comprise a group of heterogeneous disorders sharing specific characteristics such as one or more cytopenias, myeloid, erythroblastic or megakaryocytic lineage dysplasia, bone marrow failure, and a higher risk for acute myeloid leukemia (AML) development.[1] MDS diagnosis is based on a number of tests, including bone marrow examination for the morphological evidence of dysplasia, flow cytometry, and genetic analyses.[2] In fact, both cytogenetic and molecular genetic abnormalities are recurrently detected in MDS

patients and play an important role in disease diagnosis, prognosis, and therapy selection, as well as overall patient stratification.

The widely used revised international prognostic scoring system (IPSS-R) categorizes patients into five risk categories ranging from very good to very poor.[3] Chromosome banding analysis (CBA) is the gold standard methodology in order to identify cytogenetic abnormalities and to obtain a cytogenetic score applicable to the IPSS-R. However, due to its resolution of ~5–10 Mb, CBA does not allow the detection of focal abnormalities of smaller size. Additionally, actively proliferating cells are required in order to obtain metaphases and hence, a potentially analyzable result. Further genetic methodologies have been developed and applied for the detection of whole genome cytogenetic anomalies in the context of oncohematology. In particular, single nucleotide polymorphism (SNP) array analysis allows the identification of copy number abnormalities (CNA, ie, gains, losses) with a high resolution, as well as copy-neutral loss of heterozygosity (CN-LOH). However, balanced events remain undetectable by this technology and clonality cannot be as accurately established.[4] Nevertheless, CN-LOH, large or focal CNA encompassing relevant genes represent the most recurrent and frequent abnormalities in MDS.[5,6] On the contrary, real balanced chromosomal events are observed in a minority of MDS abnormal cases. The recently published molecular international prognostic scoring system for MDS (IPSS-M) further highlights the importance of identifying specific abnormalities such as CN-LOH of the *TP53* gene located on 17p13.1 as well as *KMT2A* partial tandem duplications (*KMT2A*-PTD, previously known as *MLL*-PTD).[7] These anomalies are detectable by SNP array, whereas they are nonidentifiable by CBA. Furthermore, in contrast to CBA, SNP array is performed on DNA extracted from blood, bone marrow samples as well as trephine biopsies and consequently, it has the same advantage as any other molecular method and can also be performed when bone marrow cannot be aspirated.

SNP array was previously reported as a suitable and effective methodology to determine the cytogenetic profile of MDS patients by allowing the detection of the majority of relevant abnormalities.[8–10] Although combining both methodologies was shown to improve overall results as well as diagnostic accuracy for MDS,[11] performing both tests standardly for each newly diagnosed patient result in a particularly costly and time consuming approach, which turns out to be truly beneficial only for a small number of clinical cases. Moreover, despite the successful MDS characterization by the use of SNP array and the introduction of the technology into the diagnostic workup of MDS patients, a clear cytogenetic IPSS-R comparison and evaluation of its clinical usefulness was only performed on a small patient cohort so far.[12] Therefore, testing the feasibility and the impact on the final IPSS-R and IPSS-M scores of SNP array-based results may provide novel insights and potential refinement on patient stratification.

In this study, we analyzed 290 samples from patients with an initial diagnosis or suspicion of MDS by both CBA and SNP array analyses. It was previously repeatedly shown that alternative approaches for detection of chromosome abnormalities in MDS contribute to improve patient outcome.[9–12] Here, we aim to compare the prognostic value of CBA versus SNP array in the largest cohort of MDS patients reported so far. Notably, our cohort has no selection bias and all MDS risk categories were included. Therefore, the study aims at verifying the potential applicability of SNP array to the cytogenetic IPSS-R score by comparing results obtained from both methodologies and evaluating eventual discordances. The results of the study may provide a better understanding on the concrete impact of genomic data obtained from different methodologies on clinical scores. The importance of genetic data evaluation, which requires a high degree of expertise to ensure avoidance of result misinterpretation, will also be addressed.

2 | MATERIALS AND METHODS

2.1 | Patients

A total of 290 samples were included in the study cohort. In particular, 248 bone marrow or blood samples received in the laboratory between 2017 and 2019, from patients with a diagnosis or suspicion of MDS were retrospectively investigated by both CBA and SNP array. Additionally, clinical laboratory data were available for 42 patients and included bone marrow blast percentage, hemoglobin, platelet count as well as absolute neutrophil count and age at diagnosis (Table S1). Among this group of patients, 60% were males, the mean age at diagnosis was 62 years and the mean blast count was 4.3%.

2.2 | Chromosome banding analysis

CBA was performed on stimulated cultures following standard operating procedures. Culture setup was performed up to 48 h upon sample arrival. By the use of trypsin and Giemsa for the staining procedure, GTG-banding karyotypes were obtained. For abnormal findings, a minimum of 10 metaphases were examined via the IKAROS software by MetaSystems following the available guidelines.[13] Karyotype description was based on the International System for Human Cytogenomic Nomenclature (ISCN) 2020.[14]

2.3 | SNP array

DNA was extracted by the use of the EZ1 Advanced XL instrument (QIAGEN, Hilden, Germany) through the EZ1 DNA Blood Kit. The concentration and purity were measured by NanoDrop™ One Microvolume UV–vis Spectrophotometer (Fisher Scientific, Thermo Fisher Scientific, Waltham, Massachusetts). The SNP array platform used was the Affymetrix Cytoscan HD (Thermo Fisher Scientific), which contains 2.6 million copy number markers and ~750 000 SNPs. The technique was performed as per manufacturer protocol. The analysis was subsequently conducted within the chromosome analysis suite (ChAS) software following the available genomic array guidelines.[15]

TABLE 1 Counting chromosome aberrations toward cytogenetic IPSS-R and IPSS-M evaluation: Overview of the procedure used to count chromosome abnormalities detected by SNP array toward the final scores.

SNP array abnormality type	To include in the count	Not to include in the count	Comments
Gain/loss >5 Mb	x		When multiple gains/losses are detected on the same chromosome, these are counted as one anomaly only
Gain/loss <5 Mb detected as the sole anomaly		x	
Gains/losses <5 Mb detected within the same cell proportion as the main clone	x		To be included only when potential benign germline variations have been ruled out
CN-LOH		x	

2.4 | Counting chromosome abnormalities toward cytogenetic IPSS-R and IPSS-M evaluations

The cytogenetic IPSS-R score was standardly calculated based on the CBA result. Chromosomal abnormalities were counted according to ISCN guidelines.[14] In particular, exclusively clonal aberrations were counted. In the presence of multiple clones, each independent abnormality was counted only once.

In order to calculate the cytogenetic IPSS-R obtained by SNP array results, CN-LOHs were not taken into account. Exclusively gains and losses >5 Mb were counted, as well as those that, despite being smaller than 5 Mb, were clearly associated with the larger main clone and provided details on genomic complexity.[15] Specifically, small size anomalies detected within the same cell proportion as the main clone were considered, as long as the possibility of benign germline variations was excluded. Smaller size abnormalities (<5 Mb) present as a sole anomaly were not taken into consideration toward the final score. When multiple abnormalities were detected on the same chromosome, these were counted as one anomaly only (Table 1).

The IPSS-M was additionally calculated using the corresponding web tool for 42 patients for whom clinical data was available.[7]

2.5 | Mutation analysis

For 183 cases, mutation testing was performed by the use of next-generation sequencing (NGS) and fragment analysis. For the majority of cases, a commercially available amplicon-based NGS panel was utilized (Oncomine Myeloid Research Assay, Thermo Fisher Scientific) and 36–43 genes were tested. In particular, for the IPSS-M evaluation, 43 genes were tested (Table S2). For a minority of cases, only *TP53* gene testing was performed by an in-house custom amplicon-based panel design (Thermo Fisher Scientific). NGS libraries were sequenced on the Ion Torrent S5XL Instrument and analyzed by the software SeqPilot module SexNext version 5.0 or 5.4.0. The reference genome used was the GRCh37 (hg19) and variants were described according to the HGVS nomenclature. Fragment analysis was performed for the identification of *FLT3*-ITDs (internal tandem duplications) as well as *ASXL1* hotspot mutations (NM_015338.5 c.1900_1922del and c.1934dup), which may be not easily detectable by amplicon-based NGS.

2.6 | Fluorescent in situ hybridization (FISH) analysis

FISH was performed for specific cases in order to confirm or exclude the presence of certain relevant genes involved in rearrangements (ie, *MECOM*, *KMT2A*, *ETV6*, and *NPM1*). The test was carried out on interphase nuclei, with no culture preparation. Two-hundred nuclei were analyzed for each probe of interest.

The commercially available probes used as per manufacturer protocol were the following: CytoCell EVI1 (MECOM) Breakapart; Vysis LSI MLL Dual Color, Break Apart Rearrangement Probe; Vysis ETV6 Break Apart FISH Probe Kit; CytoCell Custom NPM1/MLF1 Fusion Probe.

3 | RESULTS

3.1 | Chromosome banding analysis

Among the 290 samples analyzed, 100 cases presented with a normal karyotype (35%) and 174 with an abnormal karyotype (60%). For two cases, the karyotypes relied on only five and four available metaphases, respectively, and were, therefore, considered as noninformative (0.7%), whereas for 14 cases, karyotyping could not be performed due to culture failure (4.8%).

Among the abnormal cases, a complex karyotype was detected in 55 samples (32%); balanced translocations and inversions, not observed in the context of complex karyotypes, were identified in eight samples (4.6%). Unbalanced structural abnormalities, not observed in the context of complex karyotypes and excluding those detected along with balanced anomalies, were identified in 55 samples (32%; Figure 1A). Among the unbalanced structural abnormalities, 17 cases had a deletion of 5q and 12 cases a deletion of 20q as sole abnormalities. In total, a loss of Y chromosome was detected as a sole abnormality in 34 samples, whereas trisomy 8 was identified in nine cases as the sole aberration.

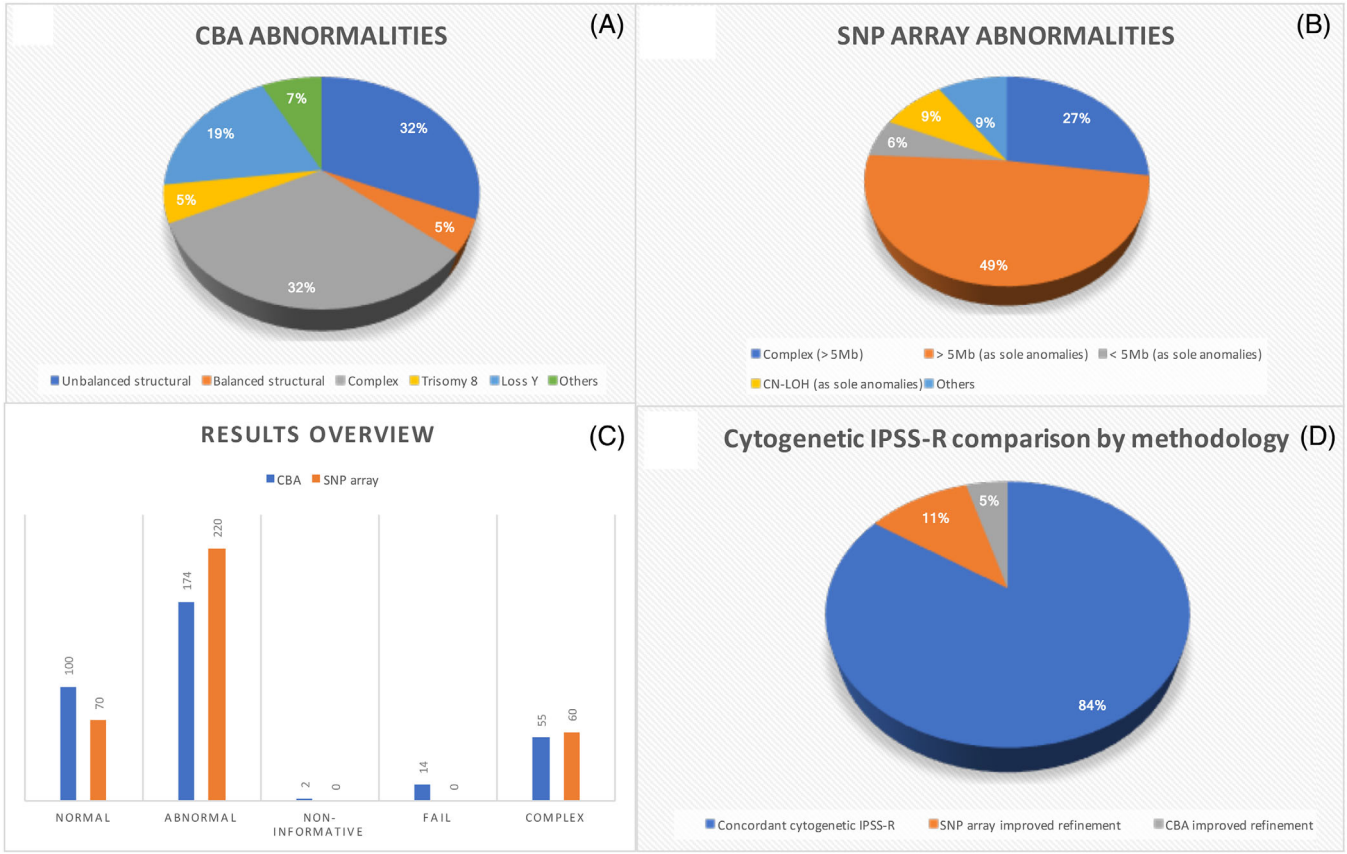

FIGURE 1 (A) Chromosome banding analysis (CBA) abnormalities: Pie chart representing the main CBA abnormality groups observed in the studied cohort. (B) Single nucleotide polymorphism (SNP) array abnormalities: Pie chart representing the main SNP array abnormality groups observed in the studied cohort. (C) Results overview: Comparison between main results obtained by CBA and SNP array. (D) Cytogenetic IPSS-R comparison by methodology: Pie chart illustrating the percentages of discordant and concordant cytogenetic IPSS-R scores calculated according to CBA and SNP array results.

3.2 | SNP array

Out of 290 samples analyzed by SNP array, 70 showed no detectable genetic abnormalities (24%), whereas 220 cases were abnormal (76%). No failed test was reported. Among the abnormal cases, 182 presented with abnormalities >5 Mb, 80 with anomalies smaller than 5 Mb and 58 cases showed the presence of CN-LOH. Additionally, anomalies larger than 5 Mb were the sole abnormalities in 108 samples; those smaller than 5 Mb were the sole anomalies in 13 samples and CN-LOH were the sole detectable cytogenetic anomalies in 19 samples (Figure 1B). Eighteen cases had abnormalities >5 Mb as well as abnormalities <5 Mb and CN-LOH.

Among the abnormal cases, CN-LOH of *TP53* was detected in 12 cases (5.5%), whereas a *KMT2A*-PTD was observed in four samples (1.8%). A complex profile was identified in 60 samples (27%). A loss of Y chromosome was detected as the sole anomaly in 33 patients (15%), while trisomy 8 was the only identifiable abnormality in nine samples (4.1%).

An overview of the main overall results obtained by CBA and SNP array is displayed in Figure 1C.

3.3 | Cytogenetic IPSS-R score

The analyzed cases were compared using the cytogenetic IPSS-R score, which was calculated according to conventional karyotype and SNP array results. Out of 290 cases, 16 could not be compared due to the lack of karyotype results or insufficient metaphases. Nevertheless, for the failed cases, the cytogenetic IPSS-R score was calculated through SNP array and shown in Table S3. Among the failed conventional karyotypes, the SNP array profile showed the following results: seven cases presented with a normal profile, one case with an abnormality smaller than 5 Mb, three cases showed the presence of a single anomaly larger than 5 Mb and three had very complex profiles (>3 abnormalities).

Among the remaining 274 cases, 245 showed a score concordance between CBA and SNP array tests (Figure 1D). Specifically, 128 cases showed a concordant good cytogenetic IPSS-R (47%), 32 cases a very good score (12%), 31 an intermediate score (11%), six cases scored as poor (2.2%), whereas 48 cases showed a very poor score (18%).

A total of 29 cases had discordant cytogenetic IPSS-R scores. In particular, when focusing on balanced rearrangements, among those

eight detected by CBA not in the context of a complex karyotype, five resulted in a discordant cytogenetic IPSS-R resulting in a downgraded cytogenetic IPSS-R array-based score (Table 2).

3.4 | International prognostic scoring system for MDS

The IPSS-M was evaluated for 42 patients for whom clinical data and molecular data were available (Table S4). In two of 42 samples, a result could not be obtained by conventional karyotype due to failure in obtaining metaphases and the cytogenetic result within the IPSS-M was therefore calculated based on SNP array data only. A discordance between the IPSS-M score calculated by the use of conventional karyotype and SNP array data was observed in five cases (Table 3).

4 | DISCUSSION

Genetic testing has a central role in MDS diagnosis, prognosis, and treatment decisions. CBA is routinely used to assess cytogenetic abnormalities and calculate the cytogenetic IPSS-R score. In this study, we firstly performed CBA and SNP array in a cohort of 290 MDS cases. Subsequently, we compared the cytogenetic IPSS-R scores obtained by both methodologies and verified its applicability to SNP array.

As previously extensively described,[16–18] the vast majority of MDS chromosomal anomalies are unbalanced rearrangements. Therefore, SNP array, with its higher resolution compared to CBA, allowed also in this study the identification of a greater number of abnormalities. Although abnormalities smaller than ~5 Mb are undetectable by CBA, they may have an impact on patient stratification. An example is represented by *KMT2A*-PTD, which is associated with an increased risk of AML transformation and a dismal overall survival.[7,19] In our cohort, *KMT2A*-PTD was confirmed to be an infrequent chromosomal event. However, it was found in four cases where CBA revealed either a normal karyotype or a deletion of 5q as the sole anomaly, therefore, providing important additional information.

Small size anomalies detected by SNP array may be challenging to interpret and should be considered according to the genomic context. They may, in fact, provide additional details on the genomic complexity when clearly part of the principal clone and may unveil the presence of unbalanced rearrangements. An example is provided in Table 2 with case N° 5, where losses on 17p and 22q, although their small sizes of <1 and 2 Mb, respectively, were retained within the final cytogenetic IPSS-R evaluation. Benign variations were excluded at the genomic location where abnormalities were detected. Additionally, the chromosomal losses were detected within the same cell proportion as that of the remaining anomalies indicating a highly likely clonal distribution. Moreover, SNP array results, used in conjunction with CBA, revealed that the translocation between chromosomes 1 and 22 was in fact unbalanced (Figure 2A–C). Similar cases may determine genomic complexity and therefore, a meticulous interpretation is required.

As per smaller focal anomalies, CN-LOH represents another relevant category of abnormalities well reported in MDS.[20] Although the majority are not included within the currently available scores for MDS, specific CN-LOH (eg, 21q CN-LOH or 4q CN-LOH) were shown to be associated with a poor survival. Notably, 17p CN-LOH encompassing *TP53* was recently included into the IPSS-M evaluation, in order to be able to identify correctly *TP53* multihit cases, which are independently and strongly associated with a poor outcome. In our cohort, 20% of cases presented with CN-LOH confirming the significant recurrence of these abnormalities in MDS. A total of 12 cases (4.1%) presented with a CN-LOH in *TP53* and all these cases were classified as multihit due to the coexistence of a *TP53* mutation detected by NGS. *TP53* CN-LOH were found in association with a complex molecular SNP array profile, which showed concordance with the complex karyotype observed by CBA. Interestingly, 17 cases of the cohort analyzed by SNP array and conventional karyotype presented CN-LOH of chromosomes other than 17p as the sole cytogenetic abnormality detected and 16 out of these harbored multiple molecular mutations in MDS-associated genes. It has been reported that CN-LOH often mask point mutations.[21] The findings in our study may indeed suggest a potential significant association between the simultaneous presence of CN-LOH and mutations, although further studies should be undertaken in order to confirm this.

SNP array, being performed on DNA, does not require actively dividing cells as a starting material. Consequently, it can be carried out on a variety of samples including bone marrow trephine biopsies and fresh tissues. In our cohort, exclusively blood and bone marrow samples were examined; however, an acceptable result could not be obtained for 16 cases by the use of CBA: 14 cases failed and two cases were not informative due to lack of metaphases. On the contrary, by the use of SNP array, an analyzable result was obtained for all 290 cases. Among the failed conventional karyotype cases, seven presented with no abnormalities by SNP array, whereas three showed a complex profile. When evaluating the cytogenetic IPSS-R for these cases, nine scored as good, one as very good, one as poor, and three as very poor. SNP array was hence, in these cases, crucial to ensure the identification and classification of high-risk patients on the genetic level and to refine the overall stratification.

The cytogenetic IPSS-R evaluation of the 274 remaining patients resulted in a concordant score for the vast majority of cases (89%). Balanced rearrangements are not detectable by SNP array and may therefore lead to divergent cytogenetic score. In our cohort, balanced abnormalities were confirmed to be infrequent events in MDS. However, five out of the eight detected cases, excluding those seen in the context of complex karyotypes, led to a discordant cytogenetic IPSS-R score. Specifically, these five discordant cases presented with a *MECOM* anomaly (either inv(3) or t(3q)) and scored, consequently, as poor by CBA, whereas they scored as either good or intermediate by SNP array since those abnormalities remained undetectable (Table 2, cases N° 22–26). Rearrangements involving *MECOM* are generally

TABLE 2 Cytogenetic IPSS-R discordant cases: list of 29 cases for which the cytogenetic IPSS-R score showed discordance between CBA and SNP array.

N°	Conventional karyotype results	SNP array results	Cytogenetic IPSS-R CC	Cytogenetic IPSS-R array	Upgraded or downgraded cytogenetic IPSS-R array-based score
1	46,XX[15]	Loss 4p15.1p15.1 (6 Mb) 90%	Good	Intermediate	Upgraded due to at least one additional abnormality detected (small size abnormality)
2	46,XX,del(11)(q22q25)[19]/46,XX[1]	Loss 11q22.3q25 80% Gain Xq27.3q28 (10 Mb) 80%	Very good	Intermediate	Upgraded due to at least one additional abnormality detected (small size abnormality)
3	45,X,-Y[3]/46,XY[17]	Loss 2p24.1p23.3 (6 Mb), Loss Y 20%	Very good	Intermediate	Upgraded due to at least one additional abnormality detected (small size abnormality)
4	46,XX,del(5)(q14q33)[8]/46,idem,del(13)(q13q21)[2]	Loss 3q26.2q26.2 (<1 Mb), 5q14.2q33.1 60% Loss 13q13.1q21.1 40%	Good	Poor	Upgraded due to at least one additional abnormality detected (small size abnormality) (*MECOM*)
5	46,XY,t(1;22)(p13;q12),t(3;5)(q25;q35.1)[13]/45,idem,-Y[6]/46,XY[2]	Loss 1p13.3, 17p11.2 (<1 Mb), 22q12.1 (<2 Mb) 90% Loss Y 10%	Poor	Very poor	Upgraded due to at least one additional abnormality detected (small size abnormality) (genomic complexity)
6	46,XX[20]	Monosomy 7 20%	Good	Poor	Upgraded due to at least one additional abnormality detected and likely proliferative disadvantage of the CC abnormal clone
7	45,X,-Y[21]/46,idem,+8[2]/46,XY[18]	Gain 3q26.1q29 40% Loss 11q22.3q25, Loss Y 40%	Intermediate	Poor	Upgraded due to at least one additional abnormality detected and likely proliferative disadvantage of the CC abnormal clone
8	45,X,-Y[9]/45,idem,+15[5]/46,XY[6]	Trisomy 12, 15, 19 30% Loss Y 30%	Intermediate	Very poor	Upgraded due to at least one additional abnormality detected and likely proliferative disadvantage of the CC abnormal clone
9	46,XXY,del(3)(p11),add(5)(q23),-12[6]/46,XXY[14]	Loss 2q35q37.3, 3p26.3p11.1, 5q23.3q34, 12p13.31p11.1, 12q12q13.11 20% Gain X 90%	Poor	Very poor	Upgraded due to at least one additional abnormality detected and likely proliferative disadvantage of the CC abnormal clone
10	45,XX,der(7;18)(q10;q10)[17]/46,XX[3]	Loss 4q22.1q25 20% Loss 7p22.3p11.1, 18p11.32p11.21 60%	Intermediate	Poor	Upgraded due to at least one additional abnormality detected and likely proliferative disadvantage of the CC abnormal clone
11	46,XY,del(11)(q14q25)[4]/46,XY[16]	Gain 3q25.31q29 20% Loss 11q14.1q25 20%	Very good	Intermediate	Upgraded due to at least one additional abnormality detected and likely proliferative disadvantage of the CC abnormal clone
12	46,XX[20]	Loss X 20%	Good	Intermediate	Upgraded due to at least one additional abnormality detected and likely proliferative disadvantage of the CC abnormal clone
13	46,XY[20]	Gain 14q11.2q32.33 (85 Mb) 30% Gain 11q23.3q23.3 (*KMTA*-PTD) 60% CN-LOH 19q13.12q13.43 30%	Good	Intermediate	Upgraded due to at least one additional abnormality detected and likely proliferative disadvantage of the CC abnormal clone
14	46,XY[20]	Loss 4p16.3p13, 4q13.3q21.1, 5q14.1q35.3, 7q21.3q36.3, 13q12.11q13.2, 13q13.3q14.3, 17p13.3p11.2, 20q12q13.2, 22q11.1q13.33 30%	Good	Very poor	Upgraded due to at least one additional abnormality detected and likely proliferative disadvantage of the CC abnormal clone (complex profile)

TABLE 2 (Continued)

N°	Conventional karyotype results	SNP array results	Cytogenetic IPSS-R CC	Cytogenetic IPSS-R array	Upgraded or downgraded cytogenetic IPSS-R array-based score
		Monosomy 15, 18, 22 30% Gain 4q11q35.2, 5p15.33p13.3, 22q11.1q12.3 30% Multiple gains/losses <5 Mb			
15	46,XY[20]	Gain 3q13.33q29, 13q31.2q32.2, 22q11.1q13.33 40% Trisomy 22 40% Loss 8p23.3p11.23, 9p23q34.3, 10q23.1q24.31, 10q25.3q26.13, 11q23.3q24.2, 15q12q15.3 Loss Y 40% Multiple gains/losses <5 Mb	Good	Very poor	Upgraded due to at least one additional abnormality detected and likely proliferative disadvantage of the CC abnormal clone (complex profile)
16	45,X,-Y[3]/46,XY[17]	CN-LOH 14q21.3q32.33 20%	Very good	Good	Upgraded due to failure to detect low level abnormality
17	45,X,-Y[9]/46,XY[11]	No abnormality detected	Very good	Good	Upgraded due to failure to detect low level abnormality
18	46,XY[20]	Loss Y 10%	Good	Very good	Downgraded due to low level abnormality detection
19	47,XY,+8[2]/46,XY[18]	CN-LOH 7q11.21q36.3 90%	Intermediate	Good	Downgraded due to failure to detect low level abnormality
20	47,XY,+8[5]/46,XY[15]	Loss 21q22.12q22.12 (<5 Mb) 70%	Intermediate	Good	Downgraded due to failure to detect low level abnormality
21	47,XY,+6[5]/46,XY[15]	No abnormality detected	Intermediate	Good	Downgraded due to failure to detect low level abnormality
22	46,XX,inv(3)(q21.3q26.2)[9]/46,idem,der(7)del(7)(p12.1p22.3)del(7)(q21.2q36.3)[4]/46,XX[7]	Loss 7p22.3p12.1, 7q21.2q36.3 20%	Poor	Intermediate	Downgraded due to failure to detect balanced rearrangements (*MECOM*)
23	46,XX,inv(3)(p?14q?12)[1]/46,idem,del(5)(q21q34)[9]	Loss 5q21.1q34 90%	Poor	Good	Downgraded due to failure to detect balanced rearrangements (*MECOM*)
24	46,XY,inv(3)(q21q26)[19]/46,XY[1]	No abnormality detected	Poor	Good	Downgraded due to failure to detect balanced rearrangements (*MECOM*)
25	46,XY,t(3;8)(q?21;q24)[12]/47,idem,+11[8]	Trisomy 11 20%	Poor	Intermediate	Downgraded due to failure to detect balanced rearrangements (*MECOM*)
26	46,XX,t(3;12)(q26;p13)[8]/46,idem,t(7;17)(q22;q11.2)[2]/46,XX[1]	Loss 17q11.2q11.2 (<5 Mb) 50%	Poor	Good	Downgraded due to failure to detect balanced rearrangements (*MECOM*)
27	46,XX,del(5)(q14q34)[3]/46,idem,?t(X;16)(p?11.2;q?12–13)[6]/46,idem,-X,-19,+2mar[6]/46,XX[5]	Loss 5q14.3q34 50%	Very poor	Good	Downgraded due to failure to detect at least one additional abnormality
28	47,XX,+X[18]/48,idem,+8[3]/47,idem,del(11)(q21q23)[2]/46,XX[1]	Trisomy X 60%	Poor	Intermediate	Downgraded due to failure to detect at least one additional abnormality

(Continues)

TABLE 2 (Continued)

N°	Conventional karyotype results	SNP array results	Cytogenetic IPSS-R CC	Cytogenetic IPSS-R array	Upgraded or downgraded cytogenetic IPSS-R array-based score
29	46,XY,del(20)(q11.2q13.1)[3]/47,idem,+Y [2]/46,XY[7]	Loss 20q11.21q13.13 20%	Intermediate	Good	Downgraded due to failure to detect at least one additional abnormality

Note: Cases are grouped according to their final downgraded or upgraded cytogenetic IPSS-R array-based score and the corresponding reason for the grading difference. Different colors are used in order to group cases according to the main reason why the score upgrade or downgrade occurred. Case N° 1: only 15 metaphases were obtained and all those available were karyotyped. Case N° 9: the additional X chromosome was not taken into account for the cytogenetic IPSS-R due to its germline origin.

TABLE 3 Discordant IPSS-M cases and failures by CBA: conventional karyotype, SNP array, NGS, and Fragment analysis results for cases for which the IPSS-M was evaluated as discordant.

Patient	Conventional karyotype result	SNP array result			NGS/Frag results	IPSS-M CC	IPSS-M SNP array
		<5 Mb	>5 Mb	CN-LOH			
1	46,XX[15]	N	Loss 4p15.1p15.1 90%	N	*SF3B1* c.2098A>G, p.Lys700Glu 46%	Very low	Low
2	46,XX[20]	N	Monosomy 7 20%	N	*RUNX1* c.493_498dup, p.Gly165_Arg166dup 15% *U2AF1* c.470A>C, p.Gln157Pro 13%	High	Very high
3	46,XX,del(11)(q22q25)[19]/46,XX[1]	N	Loss 11q22.3q25 80% Gain Xq27.3q28 80%	N	*ASXL1* c.1934dup, p.Gly646Trpfs*12 31% *JAK2* c.1849G>T, p.Val617Phe 20% *U2AF1* c.467G>A, p.Arg156His 3%	Moderate low	High
4	Fail	N	N	N	*ASXL1* c.1934dup, p.Gly646Trpfs*12 30.4% *BCOR* c.4390G>A, p.Glu1464Lys 26% *IDH2* c.419G>A, p.Arg140Gln 5.5% *SRSF2* c.284C>A, p.Pro95His 53% *STAG2* c.1840C>T, p.Arg614* 90%	NA (fail)	Very high
5	Fail	Loss 12p13.2p13.1 20%	N	N	*ETV6* c.1105C>T, p.Arg369Trp 3% *EZH2* c.2206G>A, p.Ala736Thr 22% *U2AF1* c.101C>A, p.Ser34Tyr 13%	NA (fail)	High

Note: The IPSS-M was calculated using SNP array data only for those cases where conventional karyotype failed. *N* stands for "No abnormality/mutation detected." Patient N° 1: only 15 metaphases were obtained and all those available were karyotyped.

associated with specific clinicopathologic features and may be accompanied by other genetic abnormalities including monosomy 7 or *NRAS* and *U2AF1* mutations.[22,23] They represent rare genetic rearrangements in MDS, as also shown in our study (2% cases). However, considering the significance of this genetic change and its associated prognostic impact, testing by additional methods, such as FISH, in order to ensure the identification of potential *MECOM* rearrangements, is recommended. This consideration may apply to SNP array as well as to CBA. In fact, inv(3) and t(3;3) can escape detection by GTG banding analysis if the metaphase quality is suboptimal and small deletions of chromosome 3q involving *MECOM* may be below CBA resolution and may not be detected.

A *MECOM* deletion was indeed detected exclusively by SNP array in our discordant cohort due to its size of <1 Mb. However, this resulted in a poor cytogenetic IPSS-R score by SNP array versus a good score obtained by CBA (Table 2, case N° 4).

The resolutions as well as strengths and limitations of each technique should be considered. As shown in Table 2 (cases N° 1–5), SNP array detected additional small size abnormalities compared to CBA, leading to a discordant IPSS-R. In three out of five cases, the abnormality was between 6 and 10 Mb in size, which challenges the conventional karyotype resolution depending on the chromosomal location and the banding pattern of the anomaly as well as the banding resolution obtained. In other cases, certain abnormalities were not observed by CBA despite their larger size (ie, >10 Mb) probably due to a clonal proliferative disadvantage (Table 2, cases N° 6–15). In seven out of these 10 cases, the cytogenetic IPSS-R obtained by SNP array was evaluated as poor or very poor, whereas it scored as good or intermediate by CBA. In particular, two highly complex profiles detected by SNP array showed a normal conventional karyotype. On the contrary, certain abnormal clones or subclones, although representing an overall small proportion of cells, may proliferate due to culture conditions and

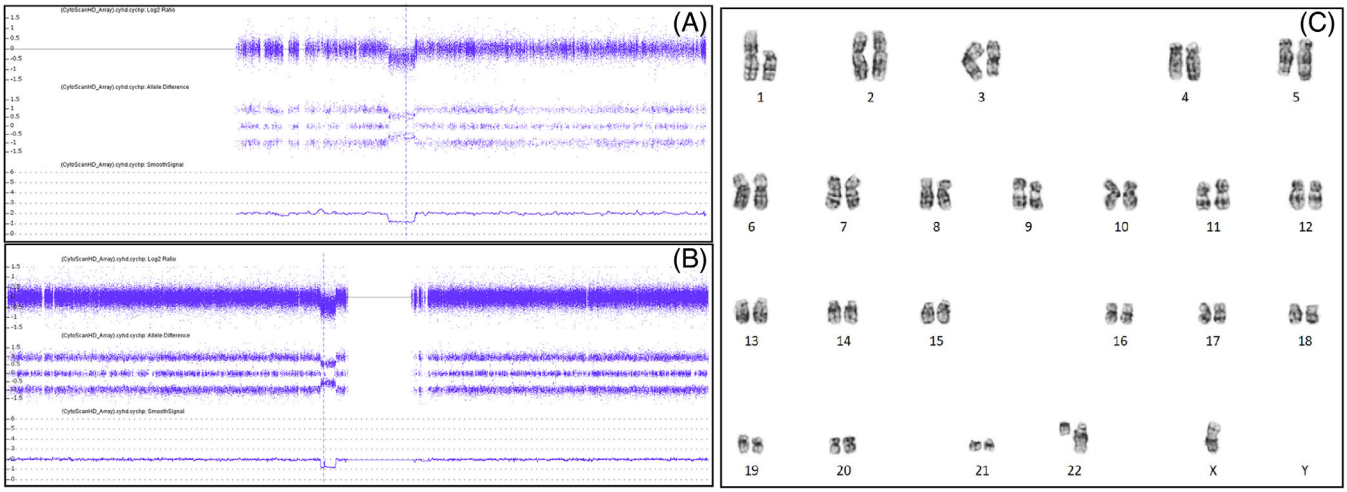

FIGURE 2 (A) Loss of chromosome arm 22q in Case 5: Data from the chromosome analysis suite (ChAS) software for Case N° 5 (Table 2), displaying the detected loss of <2 Mb in 22q associated with an unbalanced translocation between chromosomes 1 and 22. The deletion involved several probes and was not focal, with a specific structural conformation. (B) Loss of chromosome arm 1p in Case 5: Data from the ChAS software for Case N° 5 (Table 2), displaying the detected loss of 5.3 Mb in 1p associated with an unbalanced translocation between chromosomes 1 and 22. (C) Abnormal karyogram in Case 5: Conventional karyotype for Case N° 5 (Table 2) showing an apparently balanced translocation between chromosomes 1 and 22 along with other clonal aberrations. SNP array revealed that the rearrangement was in fact unbalanced.

therefore, be detected by CBA only. The complex karyotypes observed by CBA in cases N° 27 and 28 (Table 2) presented with additional anomalies, such as monosomies, marker chromosomes or a deletion, which were not identified by SNP array and consequently, led to a discordant cytogenetic IPSS-R. Hence, in these cases, CBA helped in determining clonal complexity.

Although a clonal disadvantage was observed in a number of cases analyzed by CBA, a proliferative advantage was seen in others. For instance, cases N° 16 and 17 (Table 2) presented with a loss of Y chromosome in 3 and 9 metaphases out of 20, respectively. The loss of Y chromosome may be seen in healthy individuals. However, it may also be associated with MDS since it represents one of the most common somatic genomic change in MDS hematopoietic cells in men.[24] Although it was suggested that a high proportion (ie, ≥75%) of this aberration should be considered as associated with MDS, the loss of Y chromosome often remains of delicate interpretation and the final predictive score would mostly be affected by molecular studies and by the presence of relevant mutations, in these cases. A similar consideration may also be made for cases with a low level of trisomy 8, such as cases N° 19 and 20 in Table 2, which presented trisomy 8 in two and five out of 20 metaphases, respectively, as the sole abnormality. Although being a frequently observed aberration in MDS, it may also be found in cytopenic patients in the absence of morphologic dysplasia and it should be interpreted with caution when a diagnosis is still pending.[25] Nevertheless, in cases 19 and 20 of our cohort, a CN-LOH in 7q and a small size loss of 21q were detected by SNP array, respectively, suggesting a genomic instability, which is mainly attributable to hematological malignancies such as MDS. Although SNP array added relevant information, these were not taken into account for the cytogenetic IPSS-R. Therefore, these cases scored as good by SNP array, whereas they scored as intermediate by CBA.

The recently published IPSS-M score highlights the importance of the global result, which should include the clinical, cytogenetic as well as molecular data. When evaluating the IPSS-M for 42 patients in our cohort, only five resulted in a discordant score (Table 3). In those cases, SNP array showed the presence of at least one additional cytogenetic abnormality compared to conventional karyotype, which helped in refining the final score. For all five discordant cases, the score shifted to a less favorable prognosis compared to CBA. Four of the cases had either a failed or a normal karyotype indicating that the discrepancy was related to the lack of proliferation of the abnormal clone in culture. Nevertheless, only for one case, the score difference was significant: a moderate low score obtained by CBA versus a high score obtained by SNP array (Table 3, N° 3). Among the 42 cases, eight showed no cytogenetic aberration by SNP array and CBA or by SNP array only, in case of culture failure. Therefore, molecular data were essential to ensure risk accuracy since mutations with prognostic values were identified. Furthermore, although certain cases showed a discordant result between CBA and SNP array, this divergence did not have an impact on the final IPSS-M score, suggesting the significant weight carried by molecular results in MDS.

Considering the total 29 discordant cases for the evaluated cytogenetic IPSS-R in our cohort, it can be concluded that in 13 of these cases, CBA added relevant genetic information toward a more refined score. On the other hand, for the remaining 16 cases, a more refined score resulted from SNP array results. Moreover, additional 16 cases were able to be refined by SNP array only due to the absence of an acceptable CBA result.

Although the methodologies are not equivalent, both CBA and SNP array provide the required and relevant genetic information in MDS patients. Specifically, in this study, we demonstrated the unbiased identification of chromosome alterations in contrast to CBA and successful applicability of SNP array toward the cytogenetic IPSS-R evaluation. The counting of cytogenetic abnormalities may represent a challenge regardless of the analysis method selected. In particular, SNP array data interpretation should ensure the correct distinction between potential benign variations and somatic clonal changes. Smaller size abnormalities may reveal extremely important details on genomic complexity and, if clearly part of the main clone, may be included within the final result. Challenges or borderline SNP array cases, in which a genomic complexity is highly suspected, may require a deeper investigation by the use of additional techniques. In those exceptional cases, the combination of SNP array and CBA may provide a more accurate and comprehensive result. Taking into account the progress in genetic testing, the future advent of further technologies, such as optical genome mapping or low pass and/or long read sequencing, to be used in the context of oncohematology should be considered and score evaluation may, therefore, need to be adapted to different techniques. As long as thorough and appropriate validation is performed in the laboratory demonstrating an appropriate detection sensitivity across the genome, the use of alternative cytogenetic approaches in the clinical setting may be beneficial for patient management. In this regard, it would also be beneficial if international recommendations for cytogenetic analysis in MDS would recognize the possibility of novel cytogenomic technologies as a replacement of, or an alternative for CBA. Regardless of the methodology applied, a high-quality genomic result, obtained by a deep interpretative knowledge in the field, should be provided in order to ensure refinement of risk stratification for MDS patients. Specifically, as also shown in this study, IPSS-R results are limited in capturing a comprehensive risk, whereas the recently available IPSS-M, considering global cytomolecular data, indeed provides a further and more accurate refinement in risk score for MDS patients.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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