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Impact of adjunct cytogenetic abnormalities for prognostic stratification in patients with myelodysplastic syndrome and deletion 5q

Running title: Impact of cytogenetics in MDS with deletion 5q

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ABSTRACT

This cooperative study assessed prognostic factors for overall survival (OS) and risk of transformation to acute myeloid leukaemia (AML) in 541 patients with *de novo* myelodysplastic syndrome (MDS) and deletion 5q. Additional chromosomal abnormalities were strongly related to different patients' characteristics. On multivariate analysis, the most important predictors of both OS and AML transformation risk were number of chromosomal abnormalities (P<0.001 for both outcomes), platelet count (P<0.001 and P=0.001, respectively), and proportion of bone marrow (BM) blasts (P<0.001 and P=0.016, respectively). The number of chromosomal abnormalities defined three risk categories for AML transformation (del(5q), del(5q)+1 and del(5q)+≥2 abnormalities) and two for OS (one group: del(5q) & del(5q)+1; and del(5q)+≥2 abnormalities, as the other one); with a median survival time of 58.0 and 6.8 months; respectively. Platelet count (P=0.001) and age (P=0.034) predicted OS in patients with '5q- syndrome'. This study demonstrates the importance of additional chromosomal abnormalities in MDS patients with deletion 5q, challenges the current '5q- syndrome' definition, and constitutes a useful reference series to properly analyze the results of clinical trials in these patients.

Keywords: '5q- Syndrome'; Cytogenetics; Deletion 5q; Myelodysplastic syndromes

INTRODUCTION

Myelodysplastic syndromes (MDS) are a group of clonal haematopoietic stem cell diseases characterized by dysplasia and ineffective haematopoiesis in one or more myeloid cell lines. MDS is associated with a variable overall survival (OS) and a relatively high risk of progression to acute myeloid leukaemia (AML). Evolution to AML and the clinical consequences of cytopenias are main causes of morbidity and mortality in MDS.[1-3]

Although, many specific chromosomal abnormalities have been associated with MDS, partial or complete deletion of the long arm of chromosome 5 (deletion 5q), with or without additional karyotypic abnormalities, is present in 10% to 15% of patients with *de novo* MDS, and thus is the most frequently documented recurrent cytogenetic abnormality in MDS.[4-8] Outcomes among MDS patients with deletion 5q vary greatly, both in terms of OS and risk of transformation to AML.[5,8-11] The presence of additional chromosomal abnormalities or an excess of blasts shortens OS and increases the risk of AML transformation.[5,8,10,11] The '5q- syndrome' is the only MDS group considered to represent a separate cytogenetically defined disease-category in the WHO classification. Patients with this syndrome, mostly females, are characterized by the presence of isolated deletion 5q, a blast count below 5%, favourable prognosis, and a low rate of AML transformation.[2,3] So far, no other characteristic besides the proportion of bone marrow (BM) blasts and the existence of additional chromosomal abnormalities has been recognized and universally accepted as a predictor of outcome for patients with MDS and deletion 5q.[10,11] Further, no variable has been shown to impact the clinical course of patients with WHO-

defined ‘5q- syndrome’. Lenalidomide therapy has activity in single arm clinical trials in patients with International Prognostic Scoring System (IPSS) low or intermediate-1 risk, red blood cell transfusion dependency, and deletion 5q[12-14] leading to approval by the US Food and Drug Administration for this indication. In contrast, the European Medicines Agency (EMEA) refused approval of lenalidomide for these patients, because there was no historical data against which the safety of lenalidomide could be compared, especially on concerning the expected risk of AML transformation.[15] Thus, the analysis of further prognostic parameters for OS and AML transformation in large series of MDS patients with deletion 5q is of importance.

The major aim of this global cooperation study was to assess the characteristics and natural history of a large series of 541 patients with *de novo* MDS and deletion 5q in order to identify prognostic factors of outcome.

MATERIAL AND METHODS

Patients and diagnostic criteria

Five-hundred and forty-one patients with primary MDS and deletion 5q, included in the Spanish Haematological Cytogenetic Working Group/Spanish Registry of MDS (234 patients), German-Austrian MDS Study Group (198 patients), MD Anderson Cancer Center (85 patients), Tokyo Medical University (12 patients), and other centres participating in the International Working Group on MDS Cytogenetics (12 patients) databases were the subject of this analysis. Several patients in the present study had been included in previously published reports[5,9,11] but without focusing on deletion 5q. Cases belonging to the Spanish Haematological Cytogenetic Working Group, Spanish Registry of MDS and MD Anderson were scrutinized and double-checked before inclusion for avoiding duplication.

The cases were collected between November 1972 and September 2008. The diagnosis of MDS was made according to the classification proposal of the French-American-British (FAB) study group[1]. Patients with a diagnosis of RAEB-T or CMML by FAB criteria were excluded because they are no longer considered as MDS by the WHO classification system. Whenever possible, patients were reclassified by WHO 2001 criteria.[2] Patients with an ambiguous diagnosis of MDS and those who had previously received chemotherapy or radiotherapy (therapy-related MDS) were excluded. In all patients included in this study, deletion 5q had been detected by conventional cytogenetics. The cytogenetic analysis of BM specimens was performed at the individual centres following standard chromosome-banding procedures, being crossed-validated

among centres in the previously published studies. Inclusion in the study required the analysis of at least 10 metaphases per case. The criteria defined by the International System for Human Cytogenetic Nomenclature in 2005 were used for identification of abnormal clones.[16] For example, a karyotype was considered complex when more than two independent cytogenetic abnormalities were found. When two or more clones with two aberrations were noted, the patient was categorized in the complex aberration group, whereas patients with two karyotypically independent clones with a single change in one clone and two anomalies in the second one were not considered as complex chromosomal abnormalities. Loss of Y chromosome was considered as one chromosomal abnormality. In this series, an unrelated clone was defined as a clone with cytogenetic aberrations that did not derive from the progenitor clone with the deletion 5q. The unrelated clones were considered as additional aberrations, accompanying to the deletion 5q, for the definition of its cytogenetic complexity.[17] All the cytogenetic information corresponding to the German-Austrian MDS Study Group was initially reviewed by JS and DH; and the Spanish Haematological Cytogenetic Working Group/Spanish Registry of MDS cytogenetic information, by MM, BE and FS. The final revision was done by FS, deleting those cases with incomplete cytogenetic information. The final diagnosis was provided by each institution, all of them with recognized experience in this pathology.

In keeping with the guidelines of the Declaration of Helsinki, this retrospective non-interventional study was conducted with the approval of the internal review board from the participating institutions belonging to each registry/cooperative group/centre or following individual institutional guidelines.

Prognostic factors

Different patient and disease characteristics, recorded at the time of diagnosis, were examined in the prognostic factor analysis to establish their possible relationship with OS and AML transformation. Basic demographic data included age and sex. Haematological parameters were haemoglobin level, absolute neutrophil count (ANC), platelet count, number of cytopenias, and proportion of blast cells in BM, all of them taking cut-off points and groups defined by the 1997 IPSS into account.[10] For platelet count, an additional cut-off point of 150 x 10^9/L was analyzed. Initially, we chose to test this value based on the higher platelet count that characterizes the '5q- syndrome' and the low number of patients with severe thrombocytopenia in this subset. After showing its association with prognosis in those patients, we decided to examine its potential impact in the overall series as well.

Classification systems included FAB[1] and WHO 2001[2] classifications, and IPSS scoring system. The IPSS risk categories considered were those in the original report (low, intermediate-1, intermediate-2 and high).[10] Cytogenetic findings recorded and analyzed were the presence of additional chromosome abnormalities, including the number of additional abnormalities (karyotype complexity) and the most prevalent specific additional abnormalities found (chromosome 1, chromosome 3, -7, 7q-, +8, +11, +13, 12p-, chromosome 17, -18/18q-, 20q-, +21, -X/-Y, and unrelated clones, taking into account if they were accompanying deletion 5q as a single additional chromosome abnormality or in the context of a complex karyotype), the proportion of metaphases carrying deletion 5q, and the most frequent breakpoints of the 5q deleted region (q13q31, q13q33, q22q33, q12q33, q14q34, and other breakpoints). Initially, the

number of additional chromosomal abnormalities was grouped into six categories: none (isolated deletion 5q), one, two, three, four, and five or more additional abnormalities. After showing that the clinical outcome for patients with two or more additional abnormalities was almost identical, only three cytogenetic categories were considered for all subsequent analysis: isolated deletion 5q, deletion 5q plus one additional abnormality, and deletion 5q plus two or more additional abnormalities.

Statistical analysis

Comparisons of proportions and ranks of variables between different groups were performed by Chi-square, Fisher's exact, Student-t, Mann-Whitney U or One-Way ANOVA with post-hoc Tukey's tests, as appropriate.

The Kaplan-Meier product limit method was used to estimate the probability of OS and risk of AML transformation[18-21], OS was measured from haematological diagnosis to death or last follow-up. All deaths, whether related or not to MDS, were considered as the endpoint of the follow-up interval. Patients treated with intensive AML-type chemotherapy (11 patients), haematopoietic stem cell transplantation (3 patients) or with lenalidomide (3 patients) were considered as censored data at the time of starting treatment, when the starting date of treatment was available. AML transformation was measured from diagnosis to AML development. Patients dying from any cause before developing AML were considered as censored data in the date of death for the calculation of AML transformation curves. To avoid any potential bias in the estimation of the risk of AML transformation, only patients from those registries/centres with information about AML evolution was available in most of instances, were included in the

calculation of AML transformation risk. Statistical comparisons between different actuarial curves were based on log-rank tests.[19-21]

Multivariate analysis using the Cox proportional hazards regression method for temporal events was used to identify the most significant independent prognostic variables for OS and AML transformation.[22] Characteristics selected for possible inclusion in the multivariate model where those for which there was some indication of a significant association with OS or AML transformation in the univariate analysis (Table 4), $P<0.05$. Only cases with complete data for all variables were included in the regression procedure. The forward stepwise procedure was stopped when the *P* value for entering an additional variable was above 0.05. All *P* values reported are two-sided. The selected *P* value for considering differences statistically significant in all analyses was <0.05. All analyses were performed using the statistical package SPSS version 17.0.

RESULTS

Characteristics of the patients

The overall series included 183 males (34%) and 358 females (66%) with a median age of 68 years (range, 33 – 92 years). The main characteristics of the patients at the time of diagnosis are summarized in Table 1. The median value for haemoglobin level, ANC and platelet count were 9.0 g/L (range, 2.5 – 14.0), 1.8 x 10^9/L (range, 0.10 – 38.40) and 181 x 10^9/L (range, 4 – 1,610), respectively, whereas median BM blast count was 4.0%. Most of the patients were classified as RA (49.2%) or RAEB (42.7%) according to the FAB classification; and '5q- syndrome' (39.7%), RAEB-2 (29.0%) or RAEB-1 (21.7%) by the WHO 2001 criteria.

Two-hundred and ninety-nine patients (55.3%) had deletion 5q as the sole chromosomal abnormality, 93 (17.2%) had one additional abnormality, and 149 (27.5%) had a complex karyotype with two or more associated abnormalities. The most frequent single additional anomalies to deletion 5q were del(12p) (n=11), trisomy 21 (n=10), trisomy 8 (n=9) and del(20q) (n=8). Of note, there were no patients with deletion 5q and loss of chromosome Y. However, as expected, majority of patients were females (ratio 1:2.1). In the context of complex karyotypes, aberrations most commonly found were those affecting chromosome 17 (n=40), -18/18q- (n=36), trisomy 8 (n=35), del(20q) (n=30), monosomy 7 (n=28), and involvement of chromosome 3 (n=25).

Ten of the cases included in the series (2.0%) had unrelated clones (without deletion 5q), with trisomy 8 (4 cases) and del(12p) (2 cases), being the most frequent cytogenetic aberrations.

The most common 5q deleted regions in 383 cases, in whom this information was available, were q13q33 (49.4%), q13q31 (15.9%), q22q33 (7.8%) and 20.9% other unspecific breakpoints. There was a strong correlation between the number of chromosomal abnormalities found in addition to deletion 5q and different haematological parameters, other cytogenetic findings, FAB and WHO subtype, and IPSS classification (Table 2). Comparing patients with ≥2 additional abnormalities with patients belonging to a group encompassing two cytogenetic categories [del(5q) and del(5q)+1], we observed that there were differences in sex distribution (P<0.001), and haemoglobin level between both groups (P=0.074). Platelet count and ANC showed differences between both groups (P<0.001) and a higher incidence of cytopenias as well (P<0.001). The proportion of blasts in BM was higher (P<0.001), as well as the higher proportion of cases with metaphases carrying the deletion 5q (P<0.001).

FAB and WHO diagnoses, according to the number of chromosomal abnormalities found in addition to deletion 5q, are shown in Figure 1.

Apart from differences in characteristics inherent to the definition of '5q- syndrome' (for example, absence of additional chromosomal abnormalities and lower proportion of blasts in BM), this subset of patients (n=148) had a higher median ANC (P=0.001) and median platelet count value (P<0.001) and, consequently, a lower number of cytopenias (P<0.001) than the remaining patients. Further, patients with '5q- syndrome' showed a lower median percentage of metaphases carrying deletion 5q than the rest of the patients (median 70% *versus* 90%; P<0.001) (Table 3). No significant differences in breakpoints were observed between patients with '5q- syndrome' and the remaining patients (data not shown).

Outcome and prognostic factors in the overall series

Overall survival and AML transformation data were available in 512 (94.6%) and 299 (55.3%) patients, respectively. With a median follow-up of 17.2 months (range, 1 – 326) for surviving patients, 258 patients remained alive and the median OS for the whole series was 36.8 months. Sixty-six patients evolved to AML during follow-up, with the actuarial risk of AML evolution at 5 years of 38.8%. As depicted in Table 4, univariate analysis showed that both OS and risk of AML transformation were significantly influenced by age (P<0.001 and P=0.042, respectively), sex (P<0.001 and P=0.029, respectively), ANC (P<0.001 and P=0.004, respectively) and platelet count, number of cytopenias, proportion of BM blasts, FAB and WHO subtype, IPSS risk group and number of chromosomal abnormalities found in addition to deletion 5q (P<0.001 for all variables, both OS and AML evolution), as well as the percentage of metaphases carrying deletion 5q (P<0.001 and P=0.003, respectively). Additionally, OS was shorter in those with lower haemoglobin levels (P=0.030). Different deletion breakpoints showed an impact on outcome in terms of OS (P=0.008). Although, there were one breakpoint (q22q33) that showed less median survival time, this did not differ statistically from the rest of the breakpoints (P=0.228). Figure 2 shows the actuarial curves of OS (Figure 2A) and AML transformation (Figure 2B) in the three cytogenetic groups defined according to the number of chromosomal abnormalities found in addition to deletion 5q: isolated deletion 5q, deletion 5q plus one additional abnormality, and deletion 5q plus two or more additional abnormalities. As can be appreciated, all the three aforementioned cytogenetic groups were found to have a significantly different risks of AML transformation (P<0.001 for all

comparisons) but regarding OS only two risk groups could be clearly identified, patients with deletion 5q alone or with one additional chromosomal abnormality and patients with two or more additional abnormalities. Although patients with deletion 5q plus one additional abnormality had a somewhat shorter OS than patients with isolated deletion 5q (median OS, 63.4 and 46.0 months, respectively) differences in OS among these two groups were not statistically significant (P=0.131). We were not able to determine the potential impact in the outcome of any of the additional aberrations due to the low number of cases as a single anomaly accompanying to the deletion 5q. In contrast, patients with two or more additional abnormalities showed a significantly shorter OS than the other two groups of patients (median OS, 6.8 months; P<0.001).

The same prognostic impact of the three cytogenetic groups, defined by the number of chromosomal abnormalities found in addition to deletion 5q, on OS and risk of AML transformation was evident when the analysis was restricted to patients with less than 5% and less than 10% blasts in BM (Figure 3).

As shown in Table 5, on multivariate analysis the characteristics showing an independent prognostic impact concerning OS and AML transformation risk, were the number of chromosomal abnormalities found in addition to deletion 5q (P<0.001 for both outcomes); the platelet count (P<0.001 and P=0.001, respectively); and the proportion of blasts in BM (P<0.001 and P=0.016, respectively). Age and sex also added significant prognostic information for OS (P=0.001 and P=0.020, respectively). The independent prognostic impact of platelet count in multivariate analysis was observed studying this variable both as a dichotomous and continuous one. When this variable was introduced simultaneously in the regression procedure in both ways, the dichotomized

manner was selected for entering the model. For this reason and for practical purposes all results offered are those obtained with platelet count as a dichotomized variable.

Outcome and prognostic factors in patients with '5q- syndrome'

When the analysis was restricted to 144 patients with the '5q- syndrome' diagnosis and available follow-up data, median OS was 68.8 months and actuarial risk of AML transformation at 5 years was 17.1%. On univariate analysis, male patients (median OS, 40.9 months *vs.* 80.0 months for females; *P*=0.020), patients older than 60 years of age (median OS, 45.0 months *vs.* 134.5 months for patients ≤ 60 years of age; *P*=0.005), and those with a platelet count lower than 150×10^9/L (median OS, 32.2 months *vs.* 80.0 months for patients with a platelet count greater than 150×10^9/L; *P*<0.001) had a significantly shorter OS.

Multivariate analysis showed that the main factors influencing OS were platelet count (hazard ratio [HR], 3.2; *P*=0.001) and age (HR, 2.2; *P*=0.034). None of the parameters evaluated demonstrated a significant association with AML transformation risk neither on univariate nor multivariate analysis.

Outcome and prognostic factors in patients of low and intermediate-1 risk

Patients belonging to the low and intermediate-1 IPSS category are well-known considered as good prognosis, as well as those MDS with deletion 5q. Comparing the outcomes of both groups of patients in our series, as expected, low IPSS patients has a median survival time higher than the intermediate-1 patients, though these differences were not statistically significant (58.9 months

vs. 45.0 months; *P*=0.182). The actuarial AML risk at 5 years was also similar (21.2% *vs.* 25.6%, *P*=0.437). Focusing on low risk patients, all presented isolated 5q deletion and <5% of BM blasts. The univariate analysis did not detect any prognostic factor regarding OS and AML, for those variables that there were enough patients per group. The intermediate-1 group had patients belonging to the three cytogenetic and BM blast count predefined categories. The OS univariate analysis showed the prognosis impact of cytogenetic categories (*P*=0.020), age (*P*=0.003) and platelet count (*P*=0.002). Regarding AML, cytogenetic categories (*P*=0.008) and sex (*P*=0.027) revealed their prognostic impact in the intermediate-1 subset of patients.

DISCUSSION

In this paper we present the results of a larger multicentre cooperative study that recruited the largest to-date known series of *de novo* MDS patients with deletion 5q in the pre-lenalidomide era. This has allowed us to asses the clinical characteristics, natural history and prognostic factors, with special emphasis on cytogenetic findings, being the risk of transformation to AML one of the highlights of this study. This was one of the controversial points for the approval of lenalidomide by the EMEA. Although, a phase III clinical trial comparing lenalidomide *vs.* placebo has shown some preliminary data about the risk of AML transformation in patients treated and not treated with lenalidomide[12]; herein, we have studied extensively this parameter in non treated patients, taking different prognostic factors into account.

We confirmed the strong relationship between the number of additional chromosomal abnormalities (apart from deletion 5q) and outcomes, and we are able to show that the patterns of these additional karyotype abnormalities define two distinct risk groups concerning the probability of OS and three concerning the risk of AML transformation. Platelet count and sex were the only variables independently associated with OS in a specific sub-analysis of patients with WHO-defined '5q- syndrome'.

With regard to cytogenetic abnormalities, we found that the most frequent single additional abnormalities to deletion 5q were: del(12p), trisomy 21, trisomy 8 and del(20q), the incidences of which were within the ranges reported in the literature.[23] It should be noted, however, that the number of aberrations of chromosome 7 (-7/7q-) occurring as the sole additional abnormality in this

series (n=5) was not large enough to help us to clarify its prognostic value, though a slightly non-statistically significant decrease in OS was observed in this subset of patients (data not shown).

Regarding breakpoints observed in our series, our results agree with previous studies.[9,24-26] However, some of the variability in the reported deletion breakpoints may result from the difficulties of interpretation in suboptimal chromosomal preparations and the inter-personal variability as well. For the whole series, we observed an association between the deleted regions and its outcomes, in terms of OS. Nevertheless, we did not find association of the length of the deleted segment with respect to OS. Of note, no significant differences in breakpoints were observed between patients with the '5q-syndrome' and the rest of the series, in contrast with which was previously reported.[27]

Karyotype complexity is a well-known prognostic factor in MDS.[5,8,10,11,28,29] However, in MDS patients with deletion 5q prognostic value of the number of chromosomal abnormalities in addition to deletion 5q (for example, complexity of the karyotype) is still a matter of debate, with previous reports showing conflicting results. In 2003, Stewart *et al*.[30] analyzed outcomes of haematopoietic stem cell transplants in patients with MDS or AML and deletion 5q as the sole karyotypic abnormality (n=20) *vs*. deletion 5q in combination with other chromosomal abnormalities (n=37). Overall, patients with deletion 5q as the sole karyotypic abnormality had lower rate of relapse and increased relapse-free survival. In addition to that, the blast count (<5%) was the only factor significantly associated with relapse-free survival. In 2004, Giagounidis *et al*.[9], reported a series of 76 MDS patients with deletion 5q in which those with

one additional abnormality to deletion 5q had a significant worse prognosis. However, the analysis was restricted to a subset of just 10 patients with a single additional abnormality. Recently, Holtan *et al*.[31], studying 130 deletion 5q MDS patients (including 39 with isolated deletion 5q and 16 plus one additional aberration) found similar survival for these two groups. Finally, in the largest series reported before the present one, Haase *et al*.[5] did not find statistical differences in OS between both groups of patients (82 patients with one additional abnormality out of 168 deletion 5q MDS patients). In the present enlarged series, we also failed to find a significantly different OS between patients with a sole deletion 5q (n=275) and those with a single additional abnormality (n=89), despite this latter group showed a somewhat shorter survival (46.0 vs. 63.4 months; P=0.131). Nevertheless, this similarity was not kept for the risk of AML evolution, an outcome not extensively evaluated in previous studies, as patients with a single additional abnormality showed a higher risk of evolution to AML (57.6% *vs*. 21.1% at 5 years, P<0.001). Patients with two or more additional abnormalities had a dismal prognosis in terms of OS and risk to AML transformation. The data regarding transformation to AML will be of importance, specially, in the assessment of clinical trials, a controversial point for the approval of drugs in haematological malignancies.

Multivariate analysis confirmed the independently adverse impact of the complexity of the karyotype (for instance, plus ≥2 additional aberrations) in both OS and risk of AML transformation. By contrast, differences in outcome between patients with isolated deletion 5q and those with a single additional abnormality seem not to be fully attributable to the extra aberration per se. In fact, these two groups showed significant differences in variables such as BM

blasts and platelet count (Table 2), which could account, at least in part, for the different outcomes.

Nowadays, the IPSS score[10] still being the *gold standard* for MDS stratifications and prognostication. In 2007 Malcovati *et al*. published a new scoring system based on the WHO classification, called WHO classification-based prognostic scoring system (WPSS), that includes the IPSS cytogenetic risk categories, the WHO classification, and transfusion requirements[32]. Unfortunately, this latter variable was not available in most of our patients and, thus, we were not able to evaluate the potential prognostic importance of transfusion requirements and WPSS in MDS patients with 5q deletion.

Finally, we analyzed the characteristics and outcome of 148 patients fulfilling the '5q- syndrome' WHO 2001 definition (144 with available follow-up data). WHO 2008 classification[3] restricts this diagnosis to MDS patients with isolated deletion 5q without any additional chromosomal abnormality (with the exception of a loss of the Y chromosome) and a BM blast count below 5%. Ironically, none of the patients with deletion 5q MDS in the present series showed a concurrent loss of Y chromosome, although it was observed in the context of complex karyotypes. The multivariate analysis of prognostic factors in patients with WHO 2001-defined '5q- syndrome' showed that a platelet count lower or equal to 150×10^9/L and advanced age were adversely related to OS. By contrast, none of the parameters evaluated demonstrated a significant association with AML transformation risk. This is the first series that include a large number of cases with '5q- syndrome' defined according to the WHO classification; our findings could help to a better prognostic characterization of this entity. Although, Patnaik et al., in 2010, published a large series fulfilling the current WHO-2008

definition, they were as not large as our subset of patients. However, the multivariate analysis give additional data, showing that the tranfusion need at diagnosis and dysgranulopoiesis are important prognostic factors, as well as age.[33] Additionally, they contribute with data from molecular studies, very useful in this subset of patients. They performed mutational analysis from *JAK2*, *MPL* and *IDH1* genes, which revealed mutations except for the *IDH1* gene, they are more associated with high-risk MDS or AML.[33,34]

In summary, the results of this retrospective collaborative study, which is the largest available series of patients with primary MDS and deletion 5q, most of them receiving supportive care, demonstrate the independent prognostic impact of the number of additional chromosomal abnormalities to deletion 5q, to question the currently accepted WHO definition of the '5q- syndrome'. In addition, it is the first to show the prognostic importance of platelet count and age in patients with '5q- syndrome'. Further, this series could be very useful for the design of clinical trials in MDS patients with deletion 5q. This may be of special relevance in view of the controversies arisen by the results observed in patients treated with lenalidomide.

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CONFLICT OF INTEREST

The authors declare no competing financial interests.

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FIGURE LEGENDS

Figure 1.

Incidence of the three defined cytogenetic categories (isolated del(5q), del(5q) + 1, del(5q) + ≥2) among the different morphological subtypes. *A.* According to the FAB classification. *B.* According to the WHO classification.

Abbreviations: RA, refractory anaemia; RARS, RA with ringed sideroblasts; RAEB, RA with excess of blasts; RCMD, refractory cytopenia with multilineage dysplasia; RCMD-RS, RCMD with ringed sideroblasts; MDS-U, MDS unclassifiable.

Figure 2.

Kaplan-Meier curves according to the three defined cytogenetic categories (isolated del(5q), del(5q) + 1, del(5q) + ≥2). *A.* Actuarial probability of overall survival. *B.* Cumulative probability of AML transformation.

Figure 3.

Kaplan-Meier curves according to the three defined cytogenetic categories (isolated del(5q), del(5q) + 1, del(5q) + ≥2) in patients with <5% and <10% blasts in bone marrow (BM). *A.* Actuarial probability of overall survival for patients with a BM blast count <5%. *B.* Cumulative probability of AML transformation for patients with a BM blast count <5%. *C.* Actuarial probability of overall survival for patients with a BM blast count <10%. *D.* Cumulative probability of AML transformation for patients with a BM blast count <10%.

Table 1. Patient characteristics

Characteristic	Number of patients, n (%)
Total number of patients	**541**
Age	**532**
< 60 years	129 (24.2)
≥ 60 years	403 (75.8)
Sex	**541**
Male	183 (33.8)
Female	358 (66.2)
Haemoglobin	**438**
< 10 g/dL	308 (70.3)
≥ 10 g/dL	130 (29.7)
Absolute neutrophil count	**320**
< 1.8 x 10^9/L	156 (48.8)
≥ 1.8 x 10^9/L	164 (51.2)
Platelet count	**439**
< 100 x 10^9/L	129 (29.4)
≥ 100 x 10^9/L	310 (70.6)
Cytopenias	**325**
None	48 (14.8)
One	115 (35.4)
Two	105 (32.3)
Three	57 (17.5)
BM blast count	**497**
<5 %	293 (58.8)
5-10 %	90 (18.1)
11-20 %	115 (23.1)
FAB sybtype	**508**
RA	250 (49.2)
RARS	41 (8.1)
RAEB	217 (42.7)
WHO subtype	**373**
RA	4 (1.1)
RARS	2 (0.5)
RCMD	18 (4.8)

RCMD-RS	11 (2.9)
'5q- syndrome'	148 (39.7)
RAEB-1	81 (21.7)
RAEB-2	108 (29.0)
MDS-U	1 (0.3)
Karyotype complexity	**541**
Isolated 5q-	299 (55.3)
5q- + 1 abnormality	93 (17.2)
5q- + 2 abnormalities	26 (4.8)
5q- + 3 abnormalities	21 (3.9)
5q- + 4 abnormalities	19 (3.5)
5q- + ≥5 abnormalities	83 (15.3)
Deletion 5q breakpoints	**383**
q13q31	61 (15.9)
q13q33	189 (49.4)
q22q33	30 (7.8)
q12q33	13 (3.4)
q14q34	10 (2.6)
Others	80 (20.9)
Percentage of del(5q) metaphases	**365**
<100 %	233 (63.8)
100 %	132 (36.8)
IPSS risk group	**329**
Low	89 (27.1)
Intermediate-1	110 (33.4)
Intermediate-2	83 (25.2)
High	47 (14.3)

Abbreviations: BM, bone marrow; RA, refractory anaemia; RARS, RA with ringed sideroblasts; RAEB, RA with excess of blasts; RCMD, refractory cytopenia with multilineage dysplasia; RCMD-RS, RCMD with ringed sideroblasts; MDS-U, MDS unclassifiable.

Table 2. Patient characteristics according to the karyotype complexity

	Isolated del(5q) [1]		del(5q) + 1 abnormality [2]		del(5q) + ≥2 abnormalities [3]		*P* value		
	Median (Q1-Q3)	n (%)	Median (Q1-Q3)	n (%)	Median (Q1-Q3)	n (%)	[1] *vs.* [2]	[1] *vs.* [3]	[2] *vs.* [3]
Age	**68 (59-76)**	**292**	**67 (59-76)**	**93**	**68 (59-76)**	**147**	0.772[a]	0.357[a]	0.294[a]
<60 years		77 (26.4)		24 (25.8)		28 (19.0)			
≥60 years		215 (73.6)		69 (74.2)		119 (81.0)			
Sex		**299**		**93**		**149**	0.440[b]	**<0.001**[b]	**0.031**[b]
Male		84 (28.1)		30 (32.3)		69 (46.3)			
Female		215 (71.9)		63 (67.7)		80 (53.7)			
Haemoglobin	**8.9 (2.0)***	**255**	**9.3 (1.9)***	**77**	**8.7 (1.6)***	**106**	0.327[c]	0.455[c]	0.078[c]
<10 g/dL		176 (69)		47 (61.0)		85 (80.2)			
≥10 g/dL		79 (31)		30 (39.0)		21 (19.8)			
Absolute neutrophil count	**2.2 (1.4-3.0)**	**167**	**1.6 (1.1-2.8)**	**49**	**1.1 (0.5-2.2)**	**104**	0.111[a]	**<0.001**[a]	**0.003**[a]
<1.8 x 10^9/L		58 (34.7)		25 (51.0)		73 (70.2)			
≥1.8 x 10^9/L		109 (65.3)		24 (49.0)		31 (29.8)			
Platelet count	**243 (145-377)**	**253**	**196 (106-295)**	**79**	**59 (33-113)**	**107**	**0.006**[a]	**<0.001**[a]	**<0.001**[a]
<100 x 10^9/L		35 (13.8)		18 (22.8)		76 (71.0)			
≥100 x 10^9/L		218 (86.2)		61 (77.2)		31 (29.0)			
BM blasts	**3.0 (1.0-5.0)**	**275**	**4.0 (2.0-10.0)**	**81**	**9.0 (4.0-13.0)**	**142**	**0.009**[a]	**<0.001**[a]	**<0.001**[a]
<5 %		203 (73.8)		51 (63.0)		39 (27.5)			
5-10 %		43 (15.6)		9 (11.1)		38 (26.7)			
11-20 %		29 (10.5)		21 (25.9)		65 (45.8)			
Percentage of del(5q) metaphases	**75 (52.2-100.0)**	**179**	**88.7 (64.4-100.0)**	**76**	**98.1 (69.8-100.0)**	**110**	0.227[b]	**<0.001**[b]	0.051[b]
<100 %		129 (72.1)		49 (64.5)		55 (50.0)			
100 %		50 (27.9)		27 (35.5)		55 (50.0)			
Cytopenias		**170**		**50**		**105**	0.074[d]	**<0.001**[b]	**<0.001**[d]
None		38 (22.4)		7 (14.0)		3 (2.8)			
One		79 (46.5)		21 (42.0)		15 (14.3)			
Two		46 (27.0)		15 (30.0)		44 (41.9)			
Three		7 (4.1)		3 (14.0)		43 (41.0)			

IPSS risk group	**173**	**51**	**105**	**<0.001**[d]	**<0.001**[b]	**<0.001**[b]
Low	89 (51.4)	0 (0.0)	0 (0.0)			
Intermediate-1	65 (37.6)	34 (66.7)	11 (10.5)			
Intermediate-2	18 (10.4)	15 (29.4)	50 (47.6)			
High	1 (0.6)	2 (3.9)	44 (41.9)			
FAB subtype	**277**	**85**	**146**	**0.023**[b]	**<0.001**[b]	**<0.001**[b]
RA	182 (65.7)	42 (49.4)	26 (17.8)			
RARS	21 (7.6)	11 (12.9)	9 (6.2)			
RAEB	74 (26.7)	32 (37.6)	111 (76.0)			
WHO subtype	**217**	**42**	**114**	**<0.001**[d]	**<0.001**[d]	**0.016**[d]
'5q- syndrome'	148 (68.2)	0 (0.0)	0 (0.0)			
RA	1 (0.5)	2 (4.8)	1 (0.9)			
RARS	0 (0.0)	1 (2.4)	1 (0.9)			
RCMD	3 (1.4)	8 (19.0)	7 (6.1)			
RCMD-RS	1 (0.5)	5 (11.9)	5 (4.4)			
RAEB-1	35 (16.0)	9 (21.4)	37 (32.4)			
RAEB-2	29 (13.4)	17 (40.5)	62 (54.4)			
MDS-U	0 (0.0)	0 (0.0)	1 (0.9)			

Q1, percentile 25; Q3, percentile 75; [a] Mann-Whitney U test; [b] Chi-square test; [c] One-Way ANOVA with Post-Hoc Tukey's test; [d] Fisher's exact test.

* This value corresponds to the mean and standard deviation, in brackets.

Abbreviations: BM, bone marrow; RA, refractory anaemia; RARS, RA with ringed sideroblasts; RAEB, RA with excess of blasts; RCMD, refractory cytopenia with multilineage dysplasia; RCMD-RS, RCMD with ringed sideroblasts; MDS-U, MDS unclassifiable.

Table 3. Comparative of clinical characteristics of patients with '5q- syndrome'

	'5q- syndrome'		'non 5q- syndrome'		
	Median (Q1-Q3)	**n (%)**	**Median (Q1-Q3)**	**n (%)**	***P* value**
Age	**70 (59-79)**	**147**	**67 (60-75)**	**385**	0.070[a]
<60 years		39 (26.5)		90 (23.4)	
≥60 years		108 (73.5)		295 (76.6)	
Sex		**148**		**393**	**0.035**[b]
Male		43 (29.1)		140 (35.6)	
Female		105 (70.9)		253 (64.4)	
Haemoglobin	**9.0 (1.9)***	**133**	**8.9 (1.9)***	**305**	0.420[c]
<10 g/dL		92 (69.2)		216 (70.8)	
≥10 g/dL		41 (30.8)		89 (29.2)	
Absolute neutrophil count	**2.2 (1.5-3.3)**	**86**	**1.6 (0.9-2.7)**	**234**	**<0.001** [a]
$<1.8x10^9$/L		26 (30.2)		130 (55.6)	
$\geq 1.8x10^9$/L		60 (69.8)		104 (44.4)	
Platelet count	**295 (174-412)**	**130**	**138 (60-262)**	309	**<0.001**[a]
$<100x10^9$/L		13 (10.0)		116 (37.5)	
$\geq 100x10^9$/L		117 (90.0)		193 (62.5)	
BM blasts count	**2.0 (1.0-3.0)**	**141**	**6.0 (3.0-11.0)**	**357**	**<0.001**[a]
<5 %		141 (100.0)		152 (42.6)	
5-10 %		0 (0.0)		90 (25.2)	
11-20 %		0 (0.0)		115 (32.2)	
IPSS score		**89**		**240**	**<0.001**[b]
Low		70 (78.7)		19 (7.9)	
Intermediate-1		19 (21.3)		91 (37.9)	
Intermediate-2		0 (0.0)		83 (34.6)	
High		0 (0.0)		47 (19.6)	
Percentage of del(5q) metaphases	**70.0 (40.0-93.1)**	**73**	**90.0 (61.1-100.0)**	**292**	**<0.001**[b]
<100 %		60 (82.2)		173 (59.2)	
100 %		13 (17.8)		119 (40.8)	

Q1, percentile 25; Q3, percentile 75; [a] MannWhitney U test; [b] Chi-square test; [c] Student-t test.

* This value corresponds to the mean and standard deviation, in brackets.

Table 4. Results of univariate analyses of prognostic factors for OS and AML transformation in the overall series

	Overall survival				*AML transformation*			
	n (%)	**Median survival (mo)**	**Patients alive at 5 years (%)**	***P* value**	**n (%)**	**Time to 25% probability (mo)**	**Cumulative probability of AML evolution at 5 years (%)**	***P* value**
Age	**506 (93.5)**			**<0.001**	**297 (54.9)**			**0.042**
< 60 years	121 (23.9)	80.0	52.4		66 (22.2)	13.5	47.7	
≥ 60 years	385 (76.1)	33.0	28.1		231 (77.8)	41.8	36.0	
Sex	**512 (94.6)**			**<0.001**	**299 (55.3)**			**0.029**
Male	174 (34.0)	25.0	21.2		108 (36.1)	14.9	52.1	
Female	338 (66.0)	44.9	41.9		191 (63.9)	42.1	32.4	
Haemoglobin	**429 (79.3)**			**0.030**	**290 (53.6)**			0.252
<10 g/dL	302 (70.4)	35.0	33.7		200 (69.0)	22.9	41.9	
≥10 g/dL	127 (29.6)	54.5	42.7		90 (31.6)	44.2	32.8	
Absolute neutrophil count	**318 (58.8)**			**<0.001**	**285 (52.7)**			**0.004**
<1.8 x 10^9/L	155 (48.7)	15.0	17.1		136 (47.7)	13.2	47.3	
≥1.8 x 10^9/L	163 (51.3)	38.7	45.0		149 (52.3)	51.6	28.7	
Platelet count	**428 (79.1)**			**<0.001**	**290 (53.6)**			**<0.001**
<100 x 10^9/L	127 (29.7)	8.2	8.3		100 (34.5)	6.7	67.6	
≥100 x 10^9/L	301 (70.3)	47.0	57.1		190 (65.5)	48.6	30.4	
Cytopenias	**323 (59.7)**			**<0.001**	**286 (52.9)**			**<0.001**
None	47 (14.6)	65.9	53.4		44 (15.4)	NR	15.9	
One	115 (35.6)	50.9	36.1		100 (35.0)	34.5	44.8	
Two	104 (32.2)	19.7	20.5		92 (32.1)	15.0	32.2	
Three	57 (17.6)	7.9	5.2		50 (17.5)	67	76.6	
BM blast count	**479 (88.5)**			**<0.001**	**296 (54.7)**			**<0.001**
<5 %	277 (57.8)	50.9	44.3		151 (51.0)	51.1	31.3	
5-10 %	88 (18.4)	19.7	26.2		63 (21.3)	13.5	42.1	
11-20 %	114 (23.8)	11.0	12.5		82 (27.7)	8.4	55.9	
IPSS risk group	**327 (60.4)**			**<0.001**	**289 (53.4)**			**<0.001**
Low	88 (26.9)	58.9	49.1		78 (27.0)	65.0	21.2	
Intermediate-1	109 (33.3)	45.0	34.3		94 (32.5)	52.4	25.6	
Intermediate-2	83 (25.4)	13.4	15.2		74 (25.6)	9.1	65.0	
High	47 (14.4)	6.5	0.0		43 (14.9)	5.2	100.0	
FAB subtype	**488 (90.2)**			**<0.001**	**2889 (53.4)**			**<0.001**
RA	232 (47.5)	57.0	47.3		117 (40.5)	51.4	29.8	
RARS	41 (8.4)	38.9	36.1		20 (6.9)	10.8	-	
RAEB	215 (44.1)	14.9	17.3		152 (52.6)	9.7	48.1	
WHO subtype	**362 (66.9)**			**<0.001**	**255 (47.1)**			**<0.001**
'5q- syndrome'	140 (38.7)	65.9	51.3		86 (33.7)	65.0	18.2	
RA	4 (1.1)	31.6	33.3		3 (1.2)	-	100.0	

RARS	2 (0.6)	2.7	0.0		2 (0.8)	-	100.0	
RCMD	17 (4.7)	31.0	15.9		10 (3.9)	-	100.0	
RCMD-RS	11 (3.0)	20.8	16.4		6 (2.4)	4.7	100.0	
RAEB-1	79 (21.8)	18.0	20.0		62 (24.3)	15.4	30.6	
RAEB-2	108 (29.8)	10.4	13.0		85 (33.3)	8.7	63.4	
MDS-U	1 (0.3)	9.7	0.0		1 (0.4)	-	-	
Percentage of del(5q) metaphases	**353 (65.2)**			**<0.001**	**250 (46.2)**			**0.003**
<100 %	225 (63.7)	39.6	35.2		170 (68.0)	51.1	34.4	
100 %	128 (36.3)	16.2	20.1		80 (42.0)	8.4	53.4	
Deletion 5q breakpoints	**370 (68.4)**			**0.008**				0.386
q13q31	60 (16.2)	57.1	47.4		33 (15.9)	52.4	47.4	
q13q33	181 (48.9)	39.6	38.7		110 (53.2)	26.0	38.7	
q22q33	30 (8.1)	24.0	28.2		13 (6.3)	13.2	28.2	
q12q33	13 (3.5)	57.4	46.7		10 (4.8)	-	46.7	
q14q34	10 (2.7)	73.0	33.8		1 (0.5)	-	33.8	
Others	76 (20.6)	19.7	26.9		40 (19.3)	15.4	26.9	
Karyotype complexity	**512 (94.6)**			**<0.001**	**299 (55.3)**			**<0.001**
Del(5q)	275 (53.7)	63.4	50.6		160 (53.5)	65.0	21.1	
Del(5q) + 1	89 (17.4)	46.0	40.4		43 (14.4)	14.9	57.6	
Del(5q) + 2	26 (5.1)	13.9	0.0		16 (5.4)	4.7	100.0	
Del(5q) + 3	21 (4.1)	8.1	0.0		15 (5.0)	2.6	100.0	
Del(5q) + 4	19 (3.7)	7.6	0.0		13 (4.3)	3.9	100.0	
Del(5q) + ≥5	82 (16.0)	5.7	2.3		52 (17.4)	4.2	100.0	

Abbreviations: BM, bone marrow; NR: not reached; RA, refractory anaemia; RARS, RA with ringed sideroblasts; RAEB, RA with excess of blasts; RCMD, refractory cytopenia with multilineage dysplasia; RCMD-RS, RCMD with ringed sideroblasts; MDS-U, MDS unclassifiable.

Table 5. Results of multivariate analysis of prognostic factors for OS and AML transformation in the overall series

	Overall survival			AML transformation		
Variable	**Categories**	**Hazard ratio (95% CI)**	***P* value**	**Categories**	**Hazard ratio (95% CI)**	***P* value**
Karyotype complexity	del(5q) and del(5q)+1 *vs.* del(5q)+≥2	4.1 (2.9-5.7)	<0.001	del(5q) *vs.* del(5q)+1 *vs.* del(5q)+≥2	2.9 (2.0-4.1)	<0.001
Platelet count	≤150 x 10^9/L *vs.* >150 x 10^9/L	2.0 (1.5-2.8)	<0.001	≤150 x 10^9/L *vs.* >150 x 10^9/L	2.2 (1.2-3.9)	0.001
BM blasts	<5% *vs.* 5-10% *vs.* 11-20% *vs.* >20%	1.4 (1.2-1.7)	<0.001	<5% *vs.* >5%	1.4 (1.1-1.9)	0.016
Age	<60 years *vs.* ≥60 years	1.6 (1.2-2.3)	0.001	-	-	-
Sex	Female *vs.* Male	0.7 (0.5-0.9)	0.020	-	-	-

Abbreviations: CI, confidence interval; BM, bone marrow.

Figure 1A

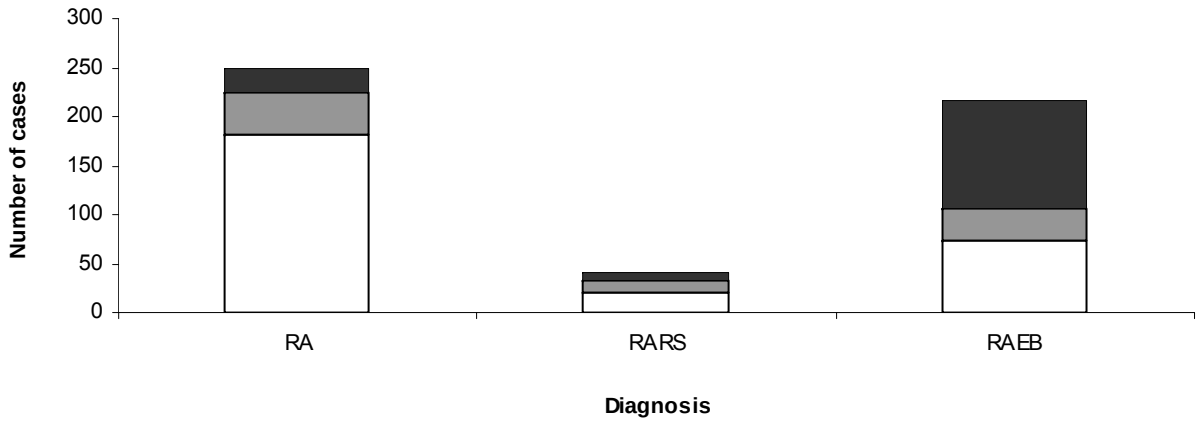


Figure 1B

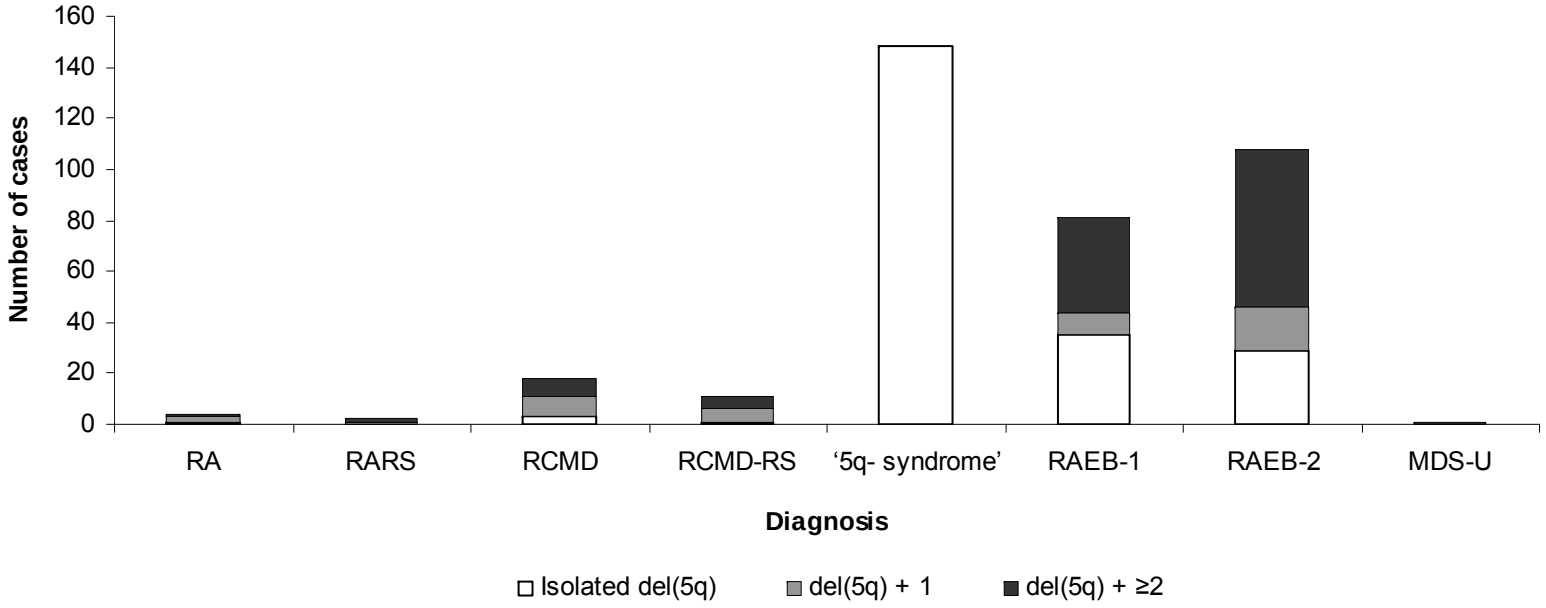


Figure 2A

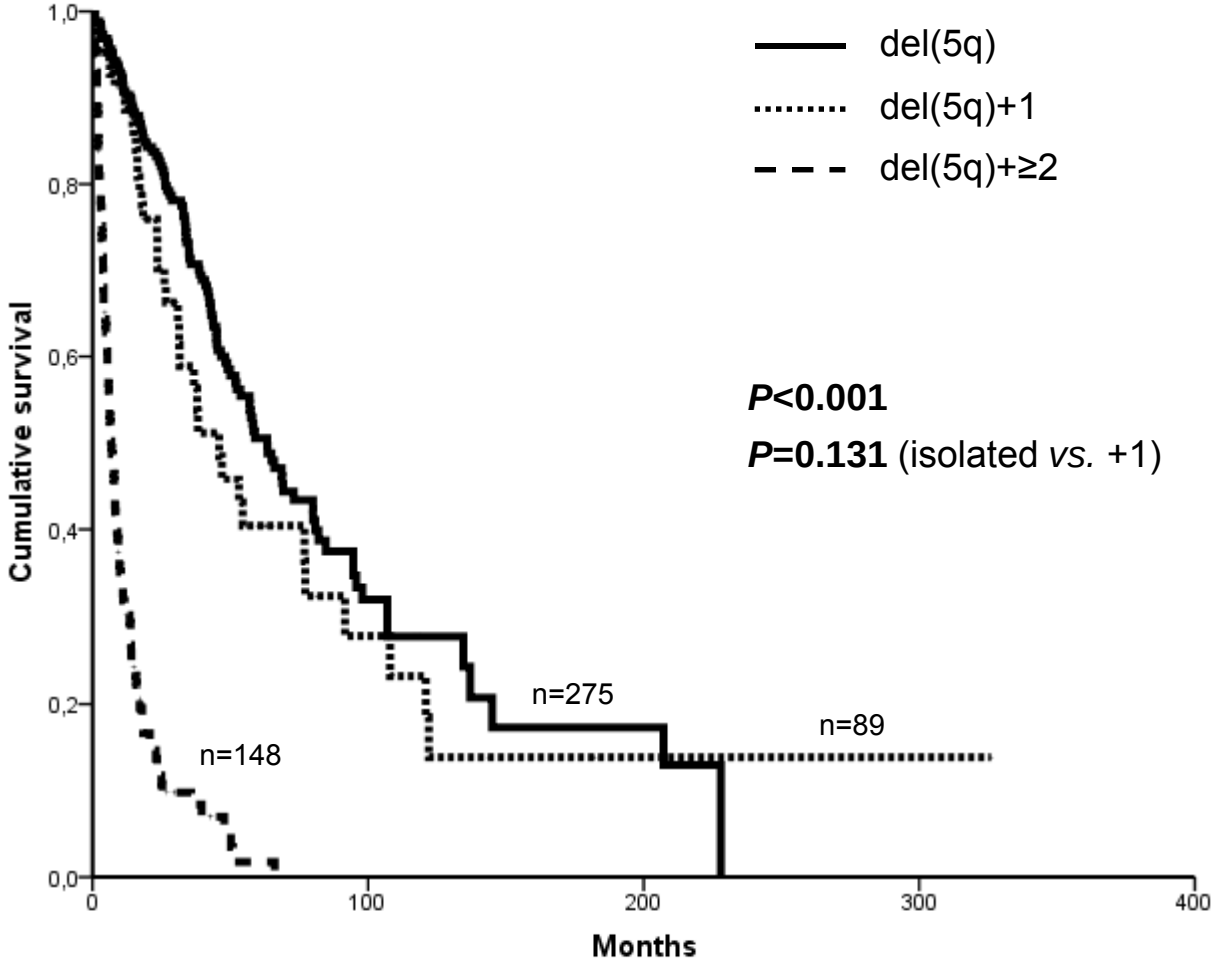

del(5q)
del(5q)+1
del(5q)+≥2
P<0.001
P=0.131 (isolated vs. +1)
n=275
n=89
n=148
Cumulative survival
Months
1,0
0,8
0,6
0,4
0,2
0,0
0
100
200
300
400

Figure 2B

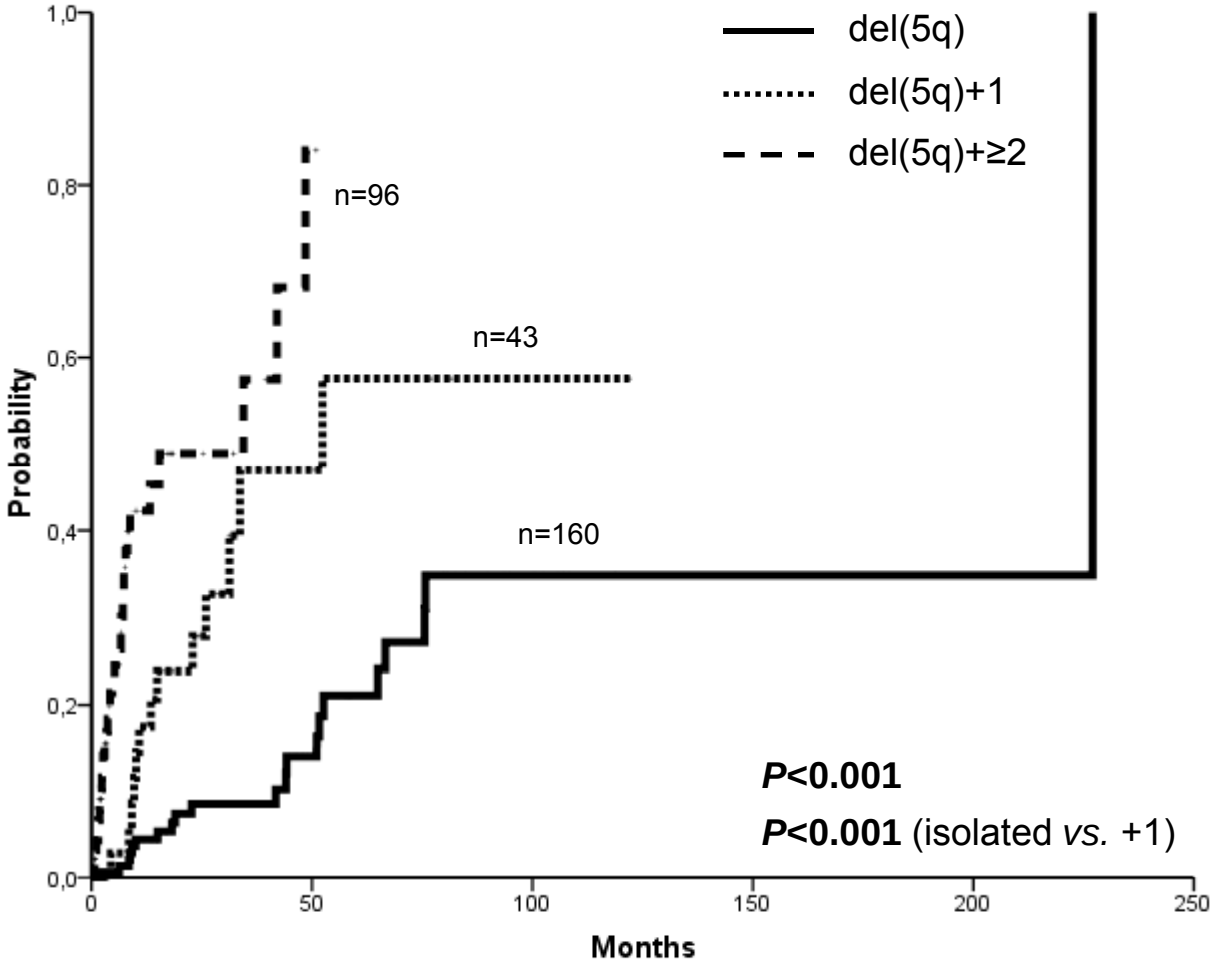

del(5q)
del(5q)+1
del(5q)+≥2
n=96
n=43
n=160
Probability
1,0
0,8
0,6
0,4
0,2
0,0
0
50
100
150
200
250
Months
P<0.001
P<0.001 (isolated vs. +1)

Figure 3A

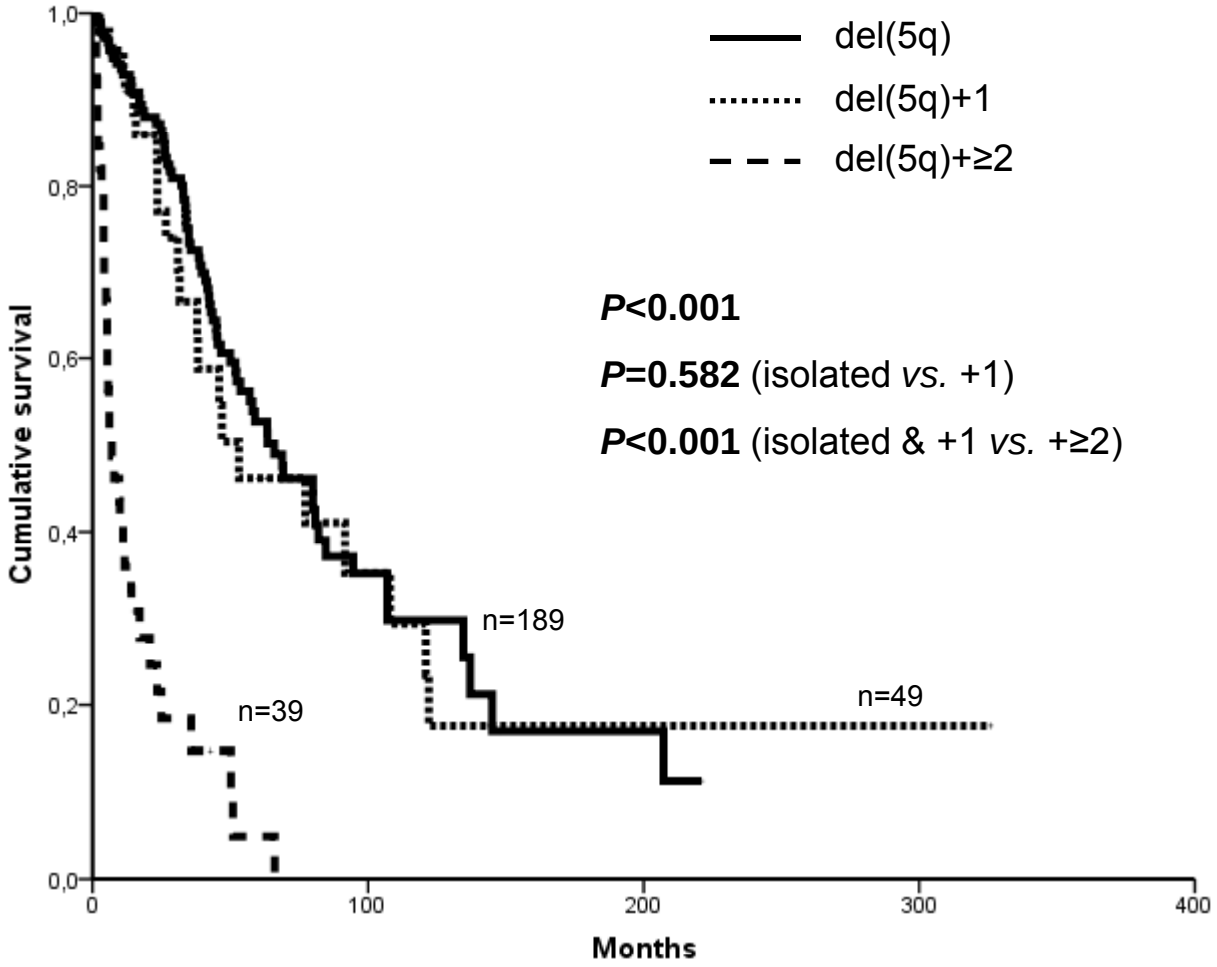

del(5q)
del(5q)+1
del(5q)+≥2
P<0.001
P=0.582 (isolated vs. +1)
P<0.001 (isolated & +1 vs. +≥2)
n=189
n=49
n=39
Cumulative survival
1,0
0,8
0,6
0,4
0,2
0,0
0
100
200
300
400
Months

Figure 3B

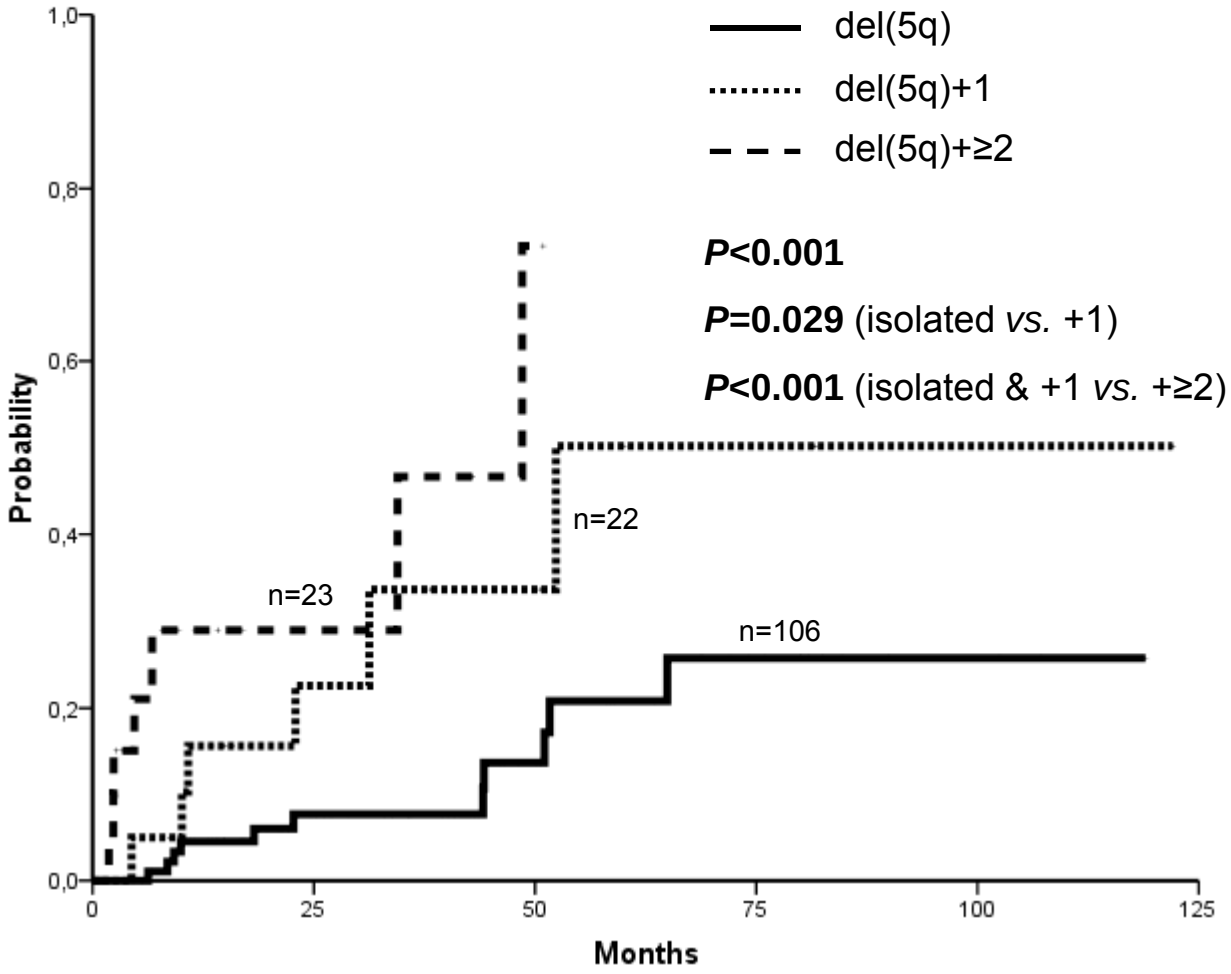

del(5q)
del(5q)+1
del(5q)+≥2
P<0.001
P=0.029 (isolated vs. +1)
P<0.001 (isolated & +1 vs. +≥2)
Probability
1,0
0,8
0,6
0,4
0,2
0,0
0
25
50
75
100
125
Months
n=23
n=22
n=106

Figure 3C

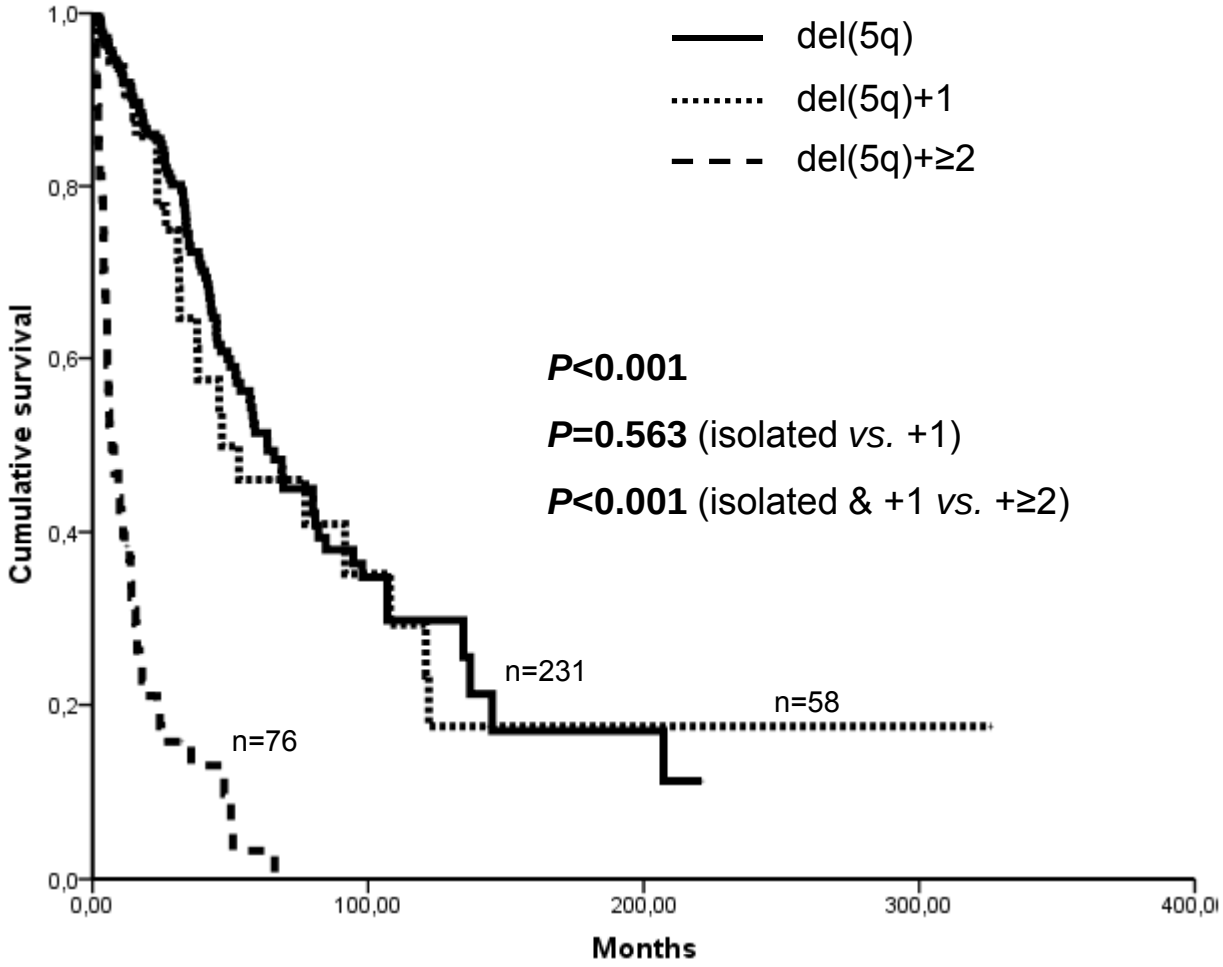
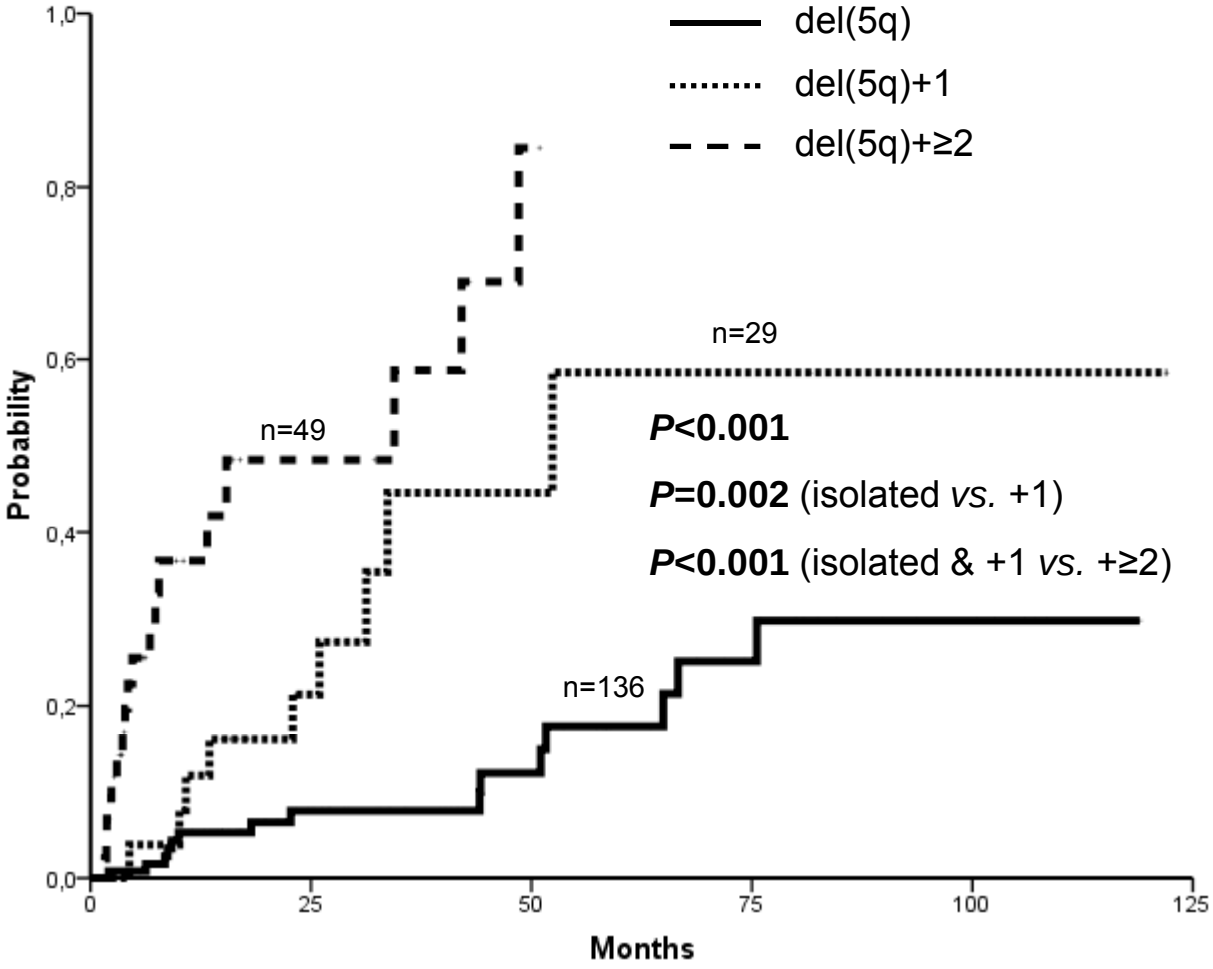

del(5q)
del(5q)+1
del(5q)+≥2
P<0.001
P=0.563 (isolated vs. +1)
P<0.001 (isolated & +1 vs. +≥2)
n=231
n=58
n=76
Cumulative survival
1,0
0,8
0,6
0,4
0,2
0,0
0,00
100,00
200,00
300,00
Months

Figure 3D


del(5q)
del(5q)+1
del(5q)+≥2
1,0
0,8
0,6
0,4
0,2
0,0
Probability
0
25
50
75
100
125
Months
n=29
n=49
n=136
P<0.001
P=0.002 (isolated vs. +1)
P<0.001 (isolated & +1 vs. +≥2)