Chromosomal analysis of myelodysplastic syndromes among atomic bomb survivors in Nagasaki

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Competing interests statement

All authors declare no competing interest in this study.

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Karyotype analysis for MDS among A-bomb survivors

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Summary

Myelodysplastic syndromes (MDS) are clonal hematopoietic disorders that develop *de novo* and also secondary to chemotherapy and / or radiation therapy. We previously demonstrated that the risk of MDS is increased among atomic bomb survivors with significant correlation to radiation dose; however, the clinical characteristics of these survivors have not been well analyzed. In this study, we investigated chromosomal abnormalities of MDS among survivors. The frequency of abnormal karyotypes was significantly higher with more very poor risk karyotypes, according to the revised International Prognostic Scoring System, among those exposed close to the hypocentre compared with unexposed cases. However, abnormal karyotype frequency did not reflect the prognosis of exposed cases with respect to distance from the hypocentre. In addition, there was no difference in prognosis between exposed and unexposed cases. Among proximally exposed cases (< 1.5 km from the hypocentre), chromosomal translocations and inversions were more frequent, and the frequency of structural alterations in chromosomes 3, 8, and 11 was significantly increased compared with unexposed cases. These results suggest that chromosomal alterations in MDS among

survivors have different features compared with those in *de novo* or therapy-related MDS. Detailed molecular study is warranted.

Introduction

Myelodysplastic syndromes (MDS) are clonal hematopoietic stem cell disorders characterized by morphological dysplasia, ineffective hematopoiesis, and transformation to acute myeloid leukaemia (AML) (Tefferi *et al*, 2009), carrying various mutated genes and pathways in almost all cases (Bejar *et al,* 2011; Haferlach *et al*, 2014). In general, 40-60% of MDS patients display clonal chromosomal abnormalities; most are unbalanced alterations, such as chromosome loss, deletions or gain, and balanced abnormalities, such as translocations, are rare (Haase *et al*, 2007). In the recently revised International Prognostic Scoring System (IPSS-R) (Greenberg *et al*, 2012), karyotype was the most influential prognostic parameter for overall survival (OS) as well as leukaemia-free survival (LFS), which was confirmed by Spanish and German groups (Valcárcel *et al*, 2013; Schanz *et al*, 2012), emphasizing its importance.

MDS arises *de novo* or is therapy-related. The outcome of patients with therapy-related MDS (T-MDS) is poorer than that of *de novo* MDS, and cytogenetic features in the two groups are partially different from each other (Mauritzson *et al*, 2002; Smith *et al*, 2003). Ionizing radiation induces chromosomal and genetic

abnormalities, and our retrospective cohort study of Nagasaki Atomic Bomb (A-bomb) survivors revealed that acute radiation exposure is associated with an increased risk of developing MDS, even 40 to 60 years after the exposure (Iwanaga *et al,* 2011). However, the successor analyses failed to demonstrate a significant difference in survival or transformation to leukaemia with respect to distance from the hypocentre, even though a higher frequency of complex karyotypes is observed among proximally exposed cases (Iwanaga *et al*, 2011; Matsuo *et al*, 2016). This raised the questions for the effect of A-bomb radiation on chromosome aberrations and its clinical meaning in MDS. This prompted us to further investigate chromosomal abnormalities and their impact on survival and leukaemia transformation in MDS among survivors in more detail.

Materials and Methods

Patients

We collected clinical information of MDS patients diagnosed from 1985 to 2013 registered in the Nagasaki-City MDS database (Iwanaga *et al*, 2011; Matsuo *et al*,

2016). This database includes information from five hospitals in Nagasaki city. Selected results, using the database, regarding the association between MDS risk and A-bomb radiation exposure and survival have been published previously (Iwanaga *et al*, 2011; Matsuo *et al*, 2016). Patients were diagnosed according to the French-American-British (FAB) classification criteria (Bennett *et al*, 1982), and their risk was evaluated using IPSS (Greenberg *et al*, 1997), and IPSS-R (Greenberg *et al*, 2012). In this analysis, we excluded patients less than 40 years old, because the youngest cases exposed to A-bomb radiation were 40 in 1985. Patients that received chemo and / or radiation therapy before the diagnosis of MDS, or who lacked successful cytogenetic data were likewise excluded. We defined A-bomb survivors as those who were present within 10 km of the hypocentre at the time of the bombing with known exposure distance. Patients were followed until June 2015. The study was conducted in accordance with the modified declaration of Helsinki, and approved by the ethic committees of the participating hospitals.

Bone marrow morphology and blood cell counts

Bone marrow morphology and blood cell count examinations were performed locally at each hospital and reviewed centrally by M.H, M.I and T.H. For clarity, MDS (and transformation to leukaemia) was classified according to the FAB classification only, because cases diagnosed before WHO classification (year of 2001) were included.

Therapy

Patients received several treatments at each institution, including supportive care with blood transfusions, antibiotics, iron chelation and haematopoietic growth factors, chemotherapies, immunosuppressive agents and hypomethylating agents. There were 7 out of 133 (see below, shown in Table 1) (5.3%), and 32 out of 269 (11.9%) cases that were treated with hypomethylating agent among exposed and unexposed patients, respectively. We excluded patients that underwent haematopoietic stem cell transplantation because no A-bomb survivors underwent such transplant therapy.

Cytogenetic examination

Cytogenetic analysis was performed using a conventional G-banding technique at the

time of first diagnosis. Cytogenetic analysis of bone marrow samples was performed at the individual centres and the results were reviewed centrally by M.H and K.Y. Karyotypes were documented according to the International System for Cytogenetic Nomenclature (ISCN) recommendations (Shaffer *et al*, 2009). The median number of metaphases in the entire cohort was 20 and ranged from 4 to 50. Only one had the metaphases less than 10. Results from fluorescent *in situ* hybridization (FISH) without conventional G-banding were excluded, and no case was tested to have normal karyotype using FISH only. The number of abnormalities was calculated according to the international guidelines (Chun *et al*, 2010). A missing chromosome was classified as monosomy; an additional chromosome as trisomy; deletions as structural losses; additions, insertions and duplications as structural gains; and balanced translocations and inversions as structurally neutral changes (Schanz *et al*, 2013).

Statistical analysis

To clarify the influence of the radiation, we categorized patients into four groups according to exposure status (exposure distance: <1.5 km, 1.5 to 2.99 km, ≥3 km in the

10 km catchment area, and unexposed patients). The estimated dose of gamma radiation was about 1 Gy at 1.5 km, and 5 mGy at 3 km from the hypocentre according to the dosimetry system 2002, and corresponding distance categories were applied in our previous studies (Young *et al*, 2005; Iwanaga *et al*, 2011). The estimated excess relative risk of MDS among survivors was 4.3/Gy (Iwanaga *et al*, 2011). Independent groups were compared using the chi-square test and Fisher's exact test. Univariate time-to-event analyses were calculated with the Kaplan-Meier method Kaplan *et al*, 1958). OS was calculated from time of first diagnosis to death or last contact, and LFS from time of diagnosis to transformation to leukaemia, or last contact without transformation. P-values for differences in time-to-event analysis were calculated by the log-rank test (Peto *et al*, 1977). Tests of significance were two sided, and P-values <0.05 were considered statistically significant. Statistical analyses were performed with Prism Version 5.0 software (Graph Pad, La Jolla, CA, USA) and EZR (Kanda 2013).

Results

Patients

In the database, 402 patients were cytogenetically evaluable; 133 patients were A-bomb survivors, and 269 were unexposed. Among survivors, the number of cases according to distance from the hypocentre was 29 in the <1.5km group (Group I), 35 in the 1.5-2.99 km group (Group II) and 69 in the ≥3 km group (Group III). The median follow-up time was 27 (0 – 330) months. In Group I, no patients were treated with DNA hypomethylating agents. Patients' characteristics are summarized in Table 1. There was no statistical difference in age, gender, FAB classification, or the percentage of blasts in bone marrow, among groups. In terms of cytopenia, the median platelet count in Group I was higher than that in the other groups. Among cases in the unexposed group, IPSS-R risk could not be calculated in 12 cases because some haematological data were not available. Prognostic risk stratification according to IPSS and IPSS-R, showed that patients in Group I tended to be stratified by IPSS as "High risk" and by IPSS-R as "Very High risk".

IPSS-R cytogenetic risk category and survival difference

Table 2 summarizes karyotype and IPSS-R cytogenetic risk category data among cases.

Comparing all A-bomb survivors and unexposed cases, there was no significant difference in the distribution of cases in each risk category. However, comparison among groups by distance from the hypocentre, the frequency of abnormal karyotypes was significantly higher in Group I than in the other groups (P = 0.006, Table 2). There was also a significant difference in the distribution of IPSS-R cytogenetic risk among all groups (P = 0.009, Table 2), with the highest frequency of "Very poor karyotype" in Group I. In the Intermediate cytogenetic risk category, the frequency of "any other independent clone" was the highest in Group I. In the Very poor cytogenetic risk group, the number of patients with extremely complex abnormalities (the number of aberrations ≥8) differed significantly among groups (27.6% in Group I, 0% in Group II, 1.4% in Group III, and 4.5% in the unexposed group, P <0.001, Table 2). There was no statistical difference in OS between all A-bomb survivors and unexposed cases (Figure 1A, P=0.731), nor in LFS (Figure 1B, P=0.294). As we reported previously (Matsuo *et al*, 2016), there was no statistical difference in either OS or LFS among Groups I, II, and III (data not shown).

Detailed Cytogenetic analysis in Group I

In general, it is assumed that those who were closer to the hypocentre received higher doses of A-bomb radiation, resulting in higher risk of haematological neoplasms. This was shown in our pervious report regarding the risk of MDS among survivors (Iwanaga *et al*, 2011). To better understand the effects of A-bomb radiation on chromosome aberration, we compared in detail the cytogenetic abnormalities between Group I and unexposed cases (Table 3). In terms of the type of chromosomal aberration in Group I, the most frequent type was deletions (Del, 13 out of 21 cases, 61.9%), followed by structural gain (St-gain, 11 cases, 52.4%), structurally neutral changes (St-neu, 10 cases, 47.6%), monosomy (7 cases, 33.3%), and trisomy (5 cases, 23.8%). St-neu, which contains random balanced translocations and inversions, was detected in 10 out of 21 cases (47.6%) without specific breakpoints, which was a significantly higher portion compared with the unexposed group (12 out of 123 cases, 9.7%, $P < 0.001$).

We next focused on individual chromosomes to see whether alterations were accumulated in specific chromosomes in Group I cases (Figure 2). Although monosomy of chromosomes 5, and 7 were observed in Group I, their frequency was not different

from that in the unexposed group (Figure 2A). Among trisomic changes, trisomy of chromosome 8 showed the highest frequency in both Group I and the unexposed group without statistically significant difference (Figure 2A). Chromosome 1 was more often involved with Del in Group I than in the unexposed group (P=0.01), and the incidence of Del in chromosomes 5 and 20 was equally high in both Group I and the unexposed group (Figure 2B). St-gain in chromosome 11 was significantly increased in Group I (P=0.002, Figure 2B). The involvement of 11q23 or MDS specific abnormalities was rare. Analysis of the structural alterations of each chromosome, combining St-gain, Del, and St-neu changes but excluding monosomy and trisomy (Figure 3), showed that chromosome 11 was affected in seven out of 21 cases (33.3%) in Group I, which was significantly more frequent compared with the unexposed group (six out of 123 cases, 4.9%) (P=0.001). Chromosomes 3 and 8 were also affected significantly more often in Group I (six and three out of 21 cases, respectively) than in the unexposed group (eight and one out of 123 cases, respectively, P = 0.007, and 0.01, respectively), with 3q27 being involved in three cases. In Group I, there were two cases each with the following abnormalities; random translocation involving 3q, monosomy 9, and monosomy X.

Effect of cytogenetic abnormalities on survival in Group I

To better investigate the impact of cytogenetic alterations on OS among A-bomb survivors, a survival curve was estimated using the Kaplan-Meier method. As shown in Figure 4A and 4B, IPSS-R cytogenetic risk category was a statistically significant factor for OS for both total survivors ($P<0.001$) and unexposed cases ($P<0.001$). It also had a significant impact on OS in Group I ($P=0.015$, Supplementary figure 1).

The number of chromosomal alterations in each case showed significant impact on OS for both survivors and unexposed cases ($P<0.001$ for both, Supplementary figure 2A and 2B). Those who had eight or more aberrations had a similar survival rate with those having four to seven aberrations among unexposed (Supplementary figure 2B). In MDS of survivors, those with eight or more aberrations showed no difference in survival rate ($P=0.162$) compared to those with four to seven aberrations (black solid line and black broken line, Supplementary figure 2A). However, Group I with "Very Poor" cytogenetic abnormalities had a significantly better OS compared with that of the unexposed group ($P = 0.008$, Figure 5A). Because all Group I cases with "Very poor" cytogenetic

abnormalities had eight or more chromosomal alterations, we compared OS of cases with eight or more alterations between Group I and the unexposed group. The survival was better in Group I than in the unexposed group among these restricted cases (P = 0.01, Figure 5B). Most of these Group I cases contained balanced translocations.

Discussion

In this study, we demonstrated the importance of the karyotype as a prognostic factor for patients with MDS among A-bomb survivors. The cytogenetic risk categories of IPSS-R could stratify both exposed and unexposed MDS cases, confirming the universal significance of cytogenetics as a predicting factor for both OS and LFS in MDS. Interestingly, however, the number of chromosomal alterations did not show the same power for exposed and unexposed cases. Among complex karyotypes, the highly complex alterations, especially eight or more alterations, predicted a poorer prognosis among unexposed cases (*de novo* MDS), as expected. However, A-bomb survivors with eight or more alterations showed no difference in survival from those with four to seven

abnormalities. This could be attributable, at least in part, to the significantly higher portion of copy number neutral chromosomal alterations, such as translocations and inversions, in MDS of survivors, which would also partly explain the high incidence of abnormal karyotype in Group I. It is well known that ionizing radiation causes DNA double strand breaks, and that balanced chromosomal translocations are generated as a result (Bender *et al*, 1988). It was reported that some long-term A-bomb survivors have chromosomal alterations, usually translocations, in haematopoietic cells as a stable chromosomal change, in particular among those exposed proximally (Amenomori *et al*, 1988). In this regard, some chromosomal abnormalities among survivors might contain stable translocations generated by A-bomb radiation with presumably low or no leukaemogenic impact. This may be one of the reasons why there was no survival difference between exposed and unexposed cases in spite of the increased number of cytogenetic changes among survivors.

Another interesting finding was the accumulation of abnormalities on chromosomes 3, 8, and 11 in MDS among survivors. Among haematological malignancies, therapy-related chromosomal changes after administration of topoisomerase II inhibitors (TOPO-II-ih)

affects chromosome 11q23 as a hot spot (Dohner *et al*, 1995; Leone *et al*, 2007); therefore, we thought A-bomb radiation could work as a TOPO-II-ih. However, 11q23 was not a recurrent alteration site, and other sites on chromosome 11 were also affected. As shown in Figure 3, in A-bomb survivors, chromosome 11 was involved in seven out of 21 cases with chromosomal abnormalities, which was significantly higher than the rate for unexposed cases; two of them had a breakpoint at 11q23, three at 11q13, and the breakpoints in the other two were located in the short arm of chromosome 11. For chromosome 3, 3q27 was affected in three out of six cases. Considering the size of chromosomes, and the random manner of DNA damage by ionizing radiation, chromosome 1 has the highest probability of being affected; however, our data does not support this hypothesis. It is suggested that selection advantage existed for haematopoietic cells that obtained chromosome 3 and / or 11 abnormalities after exposure to radiation. Molecular studies will address these questions.

In this study, the number of MDS cases among survivors was not sufficient to establish new cytogenetic risk categories, and this also contributed to the low power of the study. Considering the length of time between the explosion (1945) and when we recognized

MDS among survivors around 1980, it is difficult to collect large numbers of MDS cases among survivors, but we could confirm the usefulness of IPSS-R cytogenetic risk categories and the IPSS-R score itself to predict survival for exposed cases. Clinically, we can apply these systems to MDS among survivors.

After chemotherapy and / or radiation therapy for malignancies e.g. breast cancer, the incidence of MDS increases (Malmgren *et al*, 2016), which is called therapy-related MDS (T-MDS). A high frequency of abnormal karyotypes, especially complex karyotypes and unbalanced abnormalities of chromosomes 5, 7 and 17, are well known features induced by alkylating agents, and the 11q23 locus is frequently affected by TOPO-II-ih treatment (Mauritzson *et al*, 2002; Smith *et al*, 2003). As the exposure to A-bomb radiation is the major difference between survivors and *de novo* cases, it was assumed that MDS among survivors might have features similar to T-MDS. However, there was no apparent similarity between T-MDS and MDS among survivors in this study. First, we did not observe a significant difference in survival between exposed and unexposed groups. Second, although the frequency of abnormal karyotypes is high in proximally exposed cases, the karyotype abnormalities per se did not show a clear

similarity to those of T-MDS. It seems that detailed studies of MDS among survivors, such as molecular-based investigations, are necessary, which could provide further important answers to these questions.

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Contribution

MH collected and summarized data with SS, MM and MI, and analyzed data with K-IY, DH, and YMi. Medical data was provided by SS, KH, TJ, YT, YK, HT, SY, MT, HI, YS, JT, and YMo. Clinical diagnoses were confirmed by MH, YI and TH. K-IY and YMi designed and conducted this study, and K-IY, DH, MH, and YMi wrote the manuscript.

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Figure legends

Figure 1.

Survival of MDS patients among atomic bomb survivors and unexposed patients.

Overall survival (A) and leukaemia-free survival (B).

Figure 2.

Alteration of chromosomes by the type of aberration in Group I and unexposed cases.

Trisomy and monosomy (A), and structural gain and deletion (B).

Figure 3.

Percentage of structural aberration in each chromosome in Group I and unexposed cases.

Figure 4.

Overall survival by IPSS-R risk group among MDS patients.

Atomic bomb survivors (A) and unexposed patients (B).

Figure 5.

Overall survival of Group I cases compared with unexposed patients.

Comparison of patients with “Very poor” risk karyotype (A), and patients with more than eight alterations (B).

Figure legends for supplementary figures

Supplementary figure1.

Overall survival by IPSS-R risk group of Group 1 MDS patients among atomic bomb survivors.

Supplementary figure 2.

Overall survival by the number of chromosomal alterations among MDS patients.

Atomic bomb survivors (A) and unexposed patients (B).

Table1 Clinical characteristics of patients

	A-bomb survivors total		<1.5km (Group I)		1.5-2.99km (Group II)		3km- (Group III)		Unexposed	
Charactrtistics	n	%	n	%	n	%	n	%	n	%
Total patients	133		29		35		69		269	
Sex										
Male	71	53.4	16	55.2	22	62.9	33	47.8	167	62.1
Female	62	46.6	13	44.8	13	37.1	36	52.2	102	37.9
Age,Years										
<60	10	7.5	3	10.3	4	11.4	3	4.3	54	20.1
≧60	123	92.5	26	89.7	31	88.6	66	95.7	215	79.9
Median	72		74		72		71		72	
Range	42-94		54-83		49-90		42-94		41-92	
diagnosis period										
1985-1994	19	14.3	4	13.8	6	17.1	9	13.0	29	10.8
1995-2004	72	54.1	18	62.1	19	54.3	35	50.7	114	42.4
2004-2013	42	31.6	7	24.1	10	28.6	25	36.2	126	46.8
FAB classification										
RA	91	68.4	19	65.5	25	71.4	47	68.1	192	71.4
RARS	4	3.0	0	0.0	1	2.9	3	4.3	10	3.7
RAEB	28	21.1	7	24.1	5	14.3	16	23.2	53	19.7
RAEB-t	4	3.0	2	6.9	2	5.7	0	0.0	9	3.3
CMML	6	4.5	1	3.4	2	5.7	3	4.3	5	1.9
Bone marrow blast,%										
<5	96	72.2	19	65.5	27	77.1	50	72.5	205	76.2
5-10	14	10.5	4	13.8	2	5.7	8	11.6	34	12.6
11-20	17	12.8	4	13.8	2	5.7	11	15.9	23	8.6
21-30	4	3.0	2	6.9	2	5.7	0	0.0	7	2.6
Cytopemias										
Hb, g/L										
Median	88		82		88		91		90	
Range	32-180		55-126		56-146		32-180		25-153	
ANC, ×109/L										
Median	1.4		1.35		2		1.3		1.5	
Range	0.1-31.7		0.1-31.7		0.2-7.5		0.1-6.3		0.1-24	
PLT, ×109/L										
Median	90		106		84		89		82	
Range	1-858		29-434		4-858		1-440		0-544	
Observation time, months										
Median	40		46		34		37		25	
Range	0-254		3-207		0-254		0-252		0-330	
IPSS										
Low risk	31	23.3	4	13.8	10	28.6	17	24.6	54	20.1
Intermediate 1	68	51.1	15	51.7	16	45.7	37	53.6	145	53.9
Intermediate 2	21	15.8	4	13.8	6	17.1	11	15.9	57	21.2
High risk	13	9.8	6	20.7	3	8.6	4	5.8	13	4.8
IPSS-R										
Very low	16	12.0	2	6.9	3	8.6	11	15.9	25	9.3
Low	43	32.3	6	20.7	15	42.9	22	31.9	90	33.5
Intermediate	36	27.1	10	34.5	6	17.1	20	29.0	75	27.9
High	20	15.0	3	10.3	8	22.9	9	13.0	36	13.4
Very high	18	13.5	8	27.6	3	8.6	7	10.1	31	11.5

Table 2. Distribution of cases in karyotypic risk category by IPSS-R

	A-bomb survivors (total and subgroups by distance from hypocenter)								Unexposed (n=269)	
	Total (n=133)		<1.5km (Group I, n=29)		1.5-2.99km (Group II, n=35)		3km- (Group III, n=69)			
	n	%	n	%	n	%	n	%	n	%
Karyotype										
normal	71	53.4	8	27.6	18	51.4	45	65.2	152	56.5
abnormal	62	46.6	21	72.4	17	48.6	24	34.8	117	43.5
Number of abnormalities per patient										
1	33	24.8	8	27.6	11	31.4	14	20.3	61	22.7
2	9	6.8	3	10.3	3	8.6	3	4.3	21	7.8
3	4	3.0	2	6.9	0	0.0	2	2.9	9	3.3
4-7	7	5.3	0	0.0	3	8.6	4	5.8	14	5.2
8-	9	6.8	8	27.6	0	0.0	1	1.4	12	4.5
IPSS-R cytogenetic risk category										
Very good	6	4.5	0	0.0	2	5.7	4	5.8	12	4.5
-Y	5	3.8	0	0.0	2	5.7	3	4.3	11	4.1
del(11q)	1	0.8	0	0.0	0	0.0	1	1.4	1	0.4
Good	80	60.2	9	31.0	21	60.0	50	72.5	170	63.2
normal	71	53.4	8	27.6	18	51.4	45	65.2	152	56.5
del(5q)	2	1.5	0	0.0	0	0.0	2	2.9	4	1.5
del(12p)	0	0.0	0	0.0	0	0.0	0	0.0	1	0.4
del(20q)	5	3.8	1	3.4	2	5.7	2	2.9	11	4.1
double including del(5q)	2	1.5	0	0.0	1	2.9	1	1.4	2	0.7
Intermediate	22	16.5	8	27.6	8	22.9	6	8.7	36	13.4
del(7q)	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
+8	3	2.3	0	0.0	2	5.7	1	1.4	7	2.6
i(17q)	2	1.5	0	0.0	1	2.9	1	1.4	0	0.0
+19	0	0.0	0	0.0	0	0.0	0	0.0	1	0.4
Any other independent clones	17	12.8	8	27.6	5	14.3	4	5.8	28	10.4
Poor	9	6.8	4	13.8	1	2.9	4	5.8	25	9.3
-7	0	0.0	0	0.0	0	0.0	0	0.0	4	1.5
inv(3)/t(3q)/del(3q)	3	2.3	2	6.9	0	0.0	1	1.4	4	1.5
double including -7/del(7q)	2	1.5	0	0.0	1	2.9	1	1.4	8	3.0
complex =3	4	3.0	2	6.9	0	0.0	2	2.9	9	3.3
Very poor	16	12.0	8	27.6	3	8.6	5	7.2	26	9.7
complex >3	16	12.0	8	27.6	3	8.6	5	7.2	26	9.7

Table 3. Frequency of the type of chromosomal aberration

		Group I (n=21)		Unexposed (n=123)		*p value*
		n	%	n	%	
monosomy	−	14	66.7	76	61.8	
	+	7	33.3	47	38.2	0.809
trisomy	−	16	76.2	95	77.2	
	+	5	23.8	28	22.8	0.999
deletion	−	8	38.1	57	46.3	
	+	13	61.9	66	53.7	0.636
structural gain	−	10	47.6	80	65.0	
	+	11	52.4	43	35.0	0.148
structural neutral change	−	11	52.4	111	90.2	
	+	10	47.6	12	9.8	<0.001

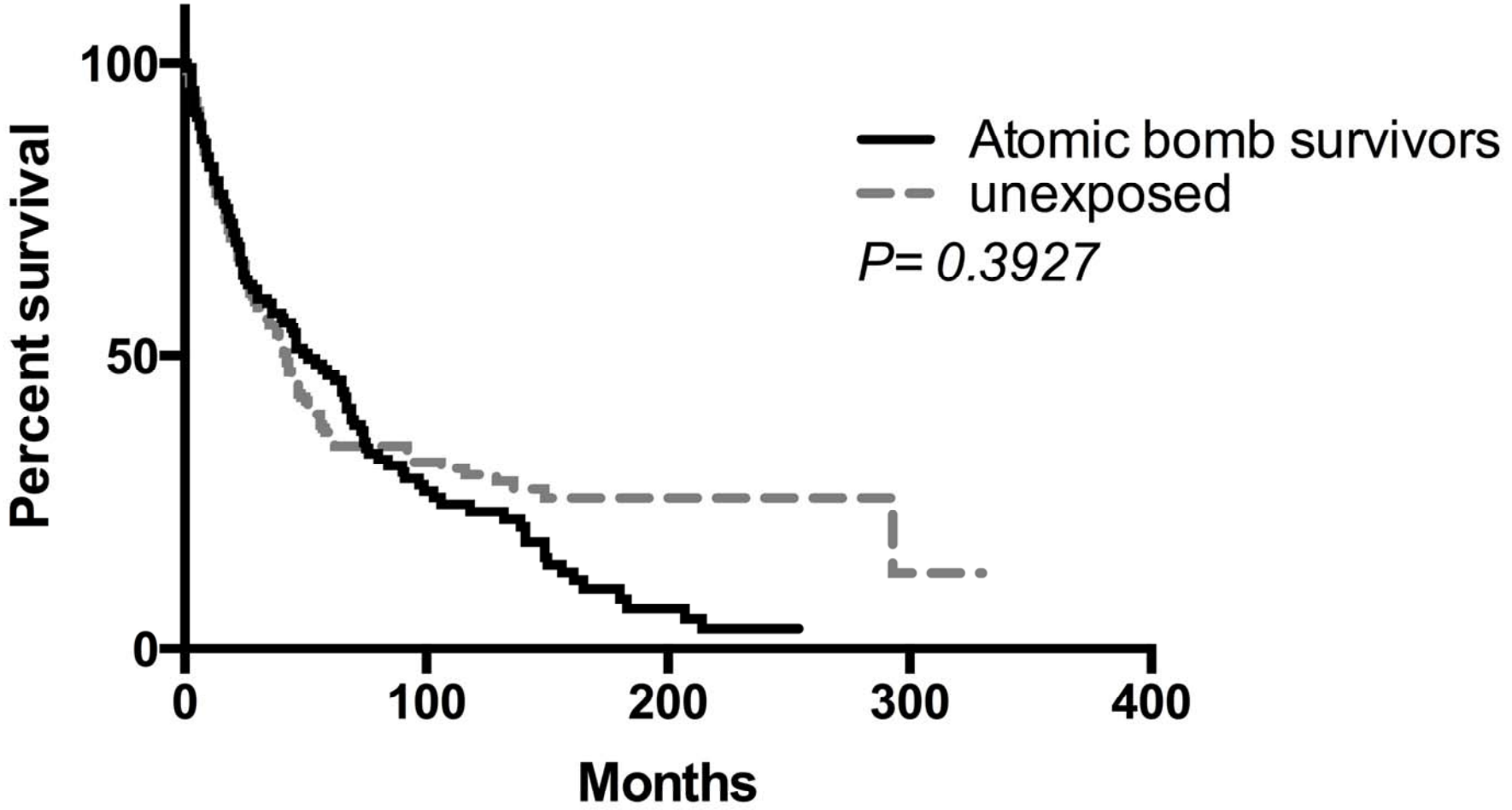

100
50
0
Percent survival
0
100
200
300
400
Months
Atomic bomb survivors
unexposed
P= 0.3927

Figure 1A

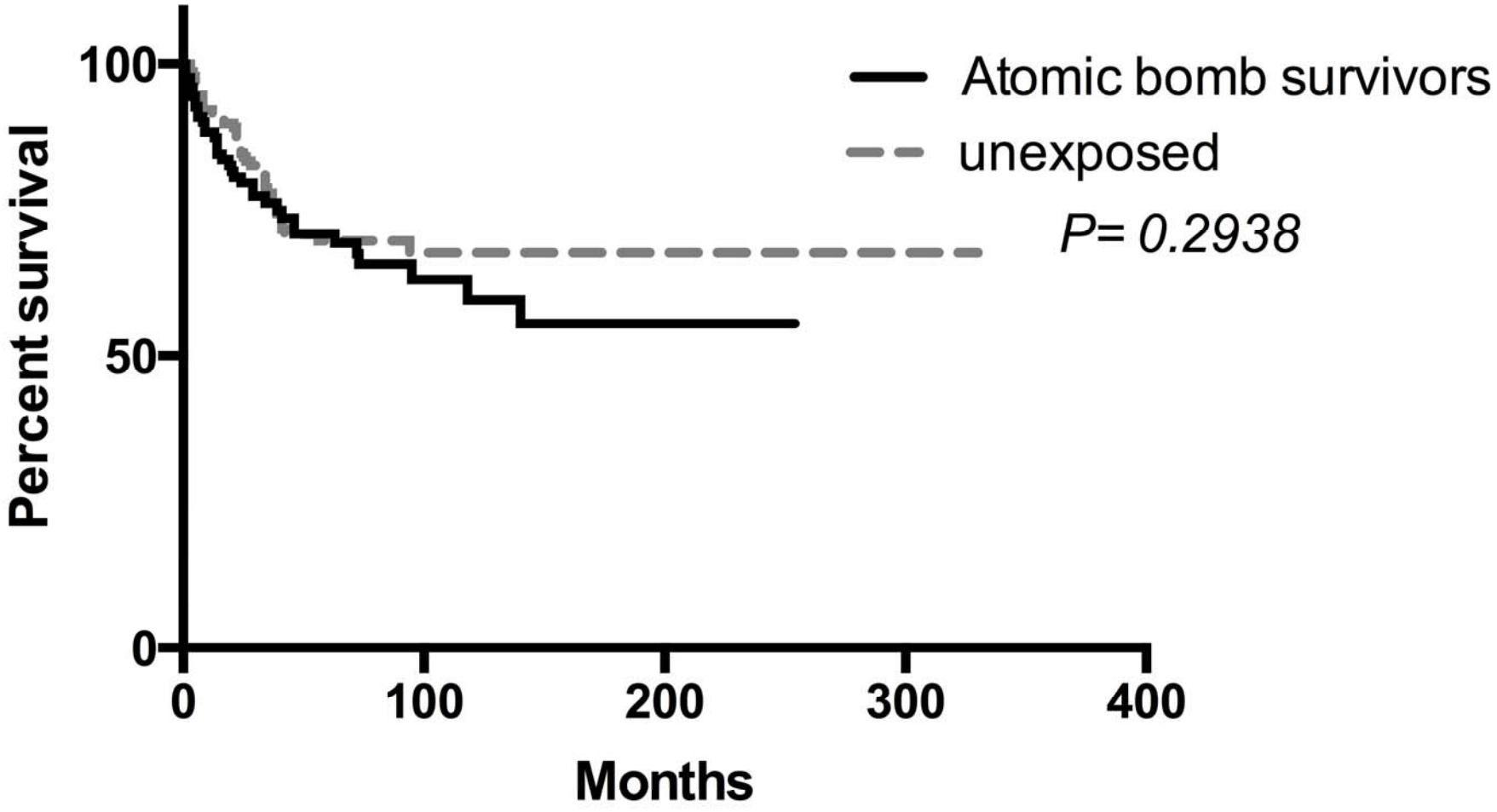

100
50
0
Percent survival
0
100
200
300
400
Months
Atomic bomb survivors
unexposed
P= 0.2938

Figure 1B

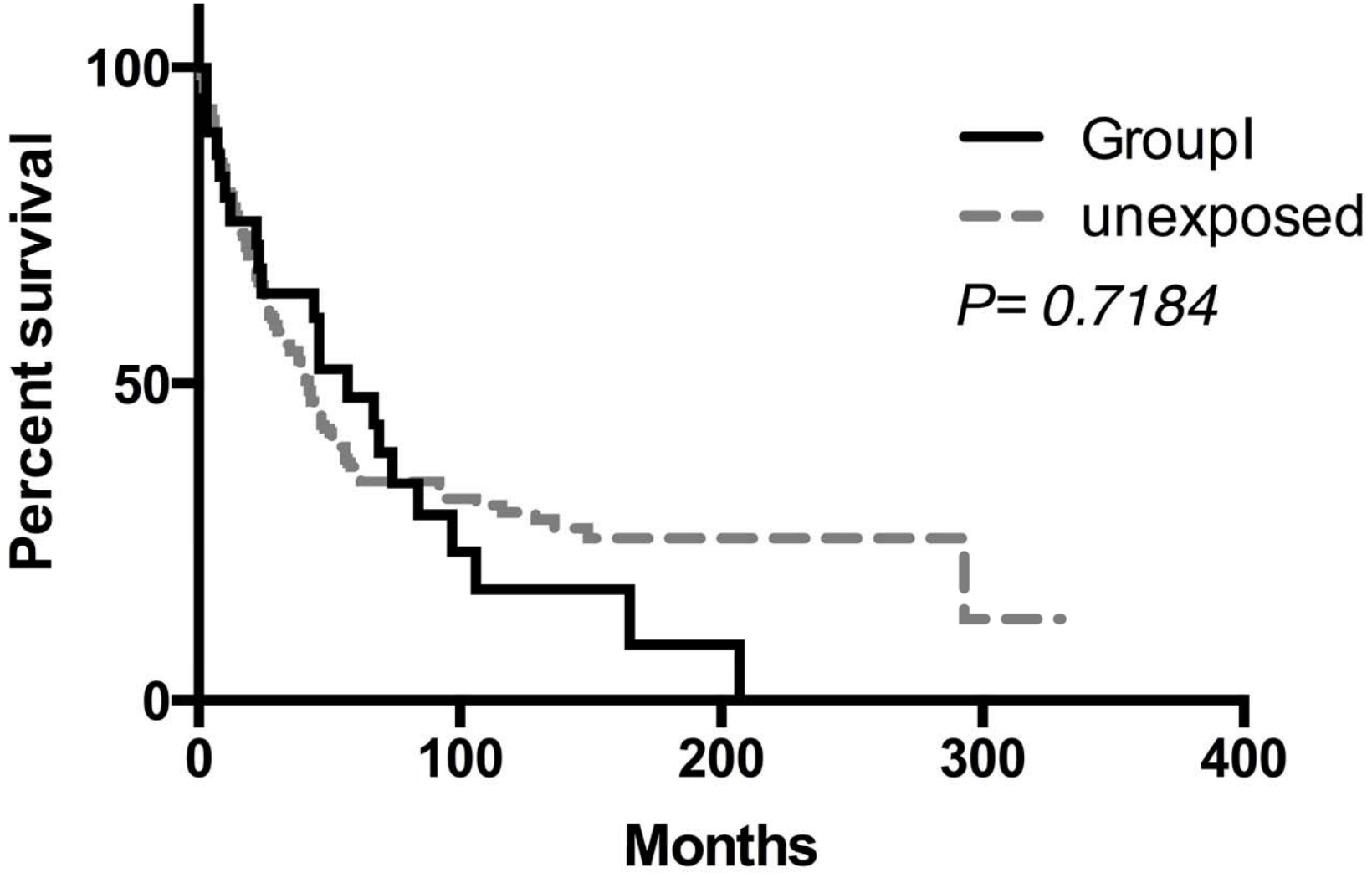

100
50
0
Percent survival
0
100
200
300
400
Months
GroupI
unexposed
P= 0.7184

Figure 1C

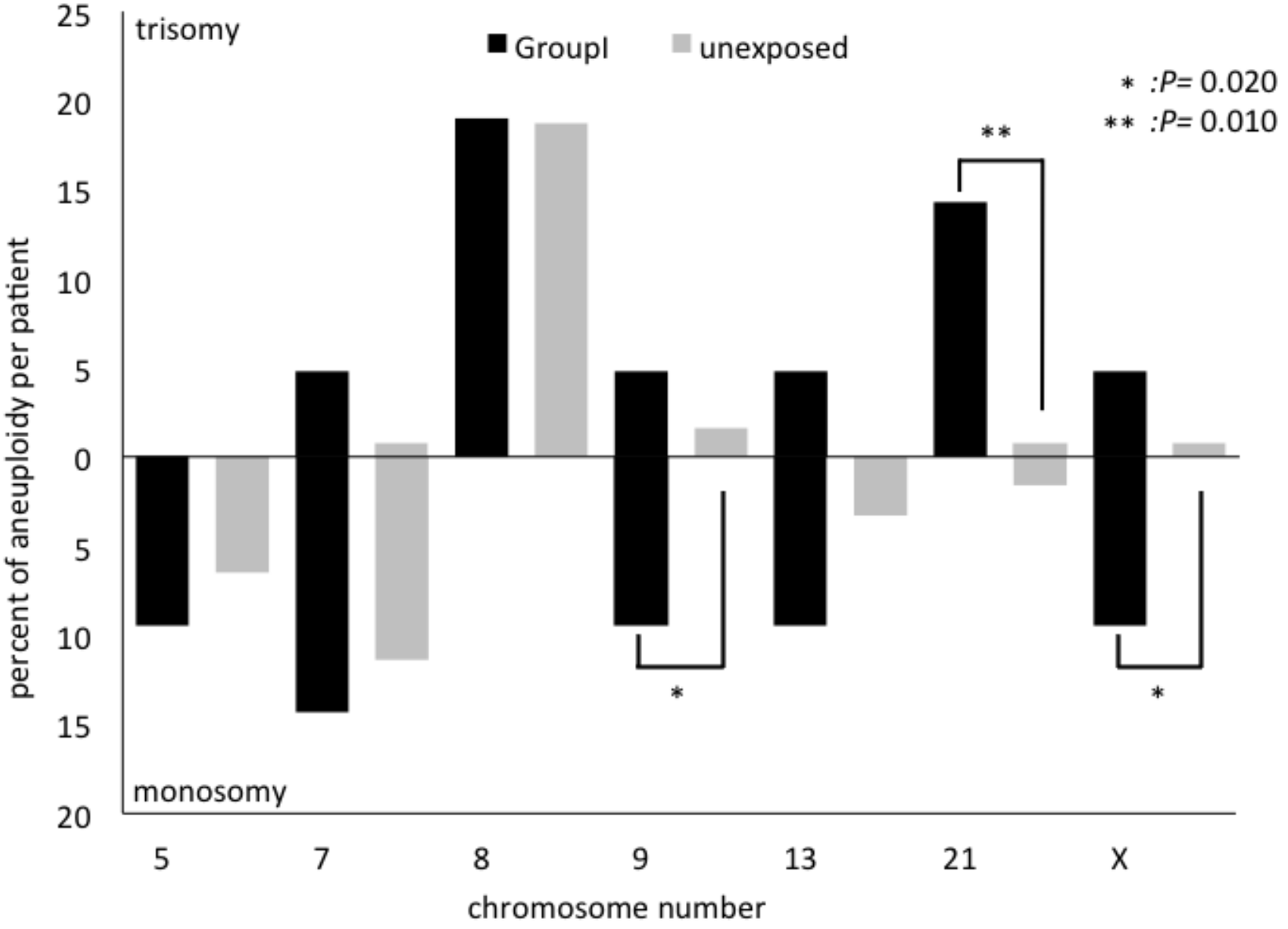

trisomy
GroupI
unexposed
* :P= 0.020
** :P= 0.010
percent of aneuploidy per patient
25
20
15
10
5
0
5
10
15
20
monosomy
**
*
*
5
7
8
9
13
21
X
chromosome number

Figure 2A

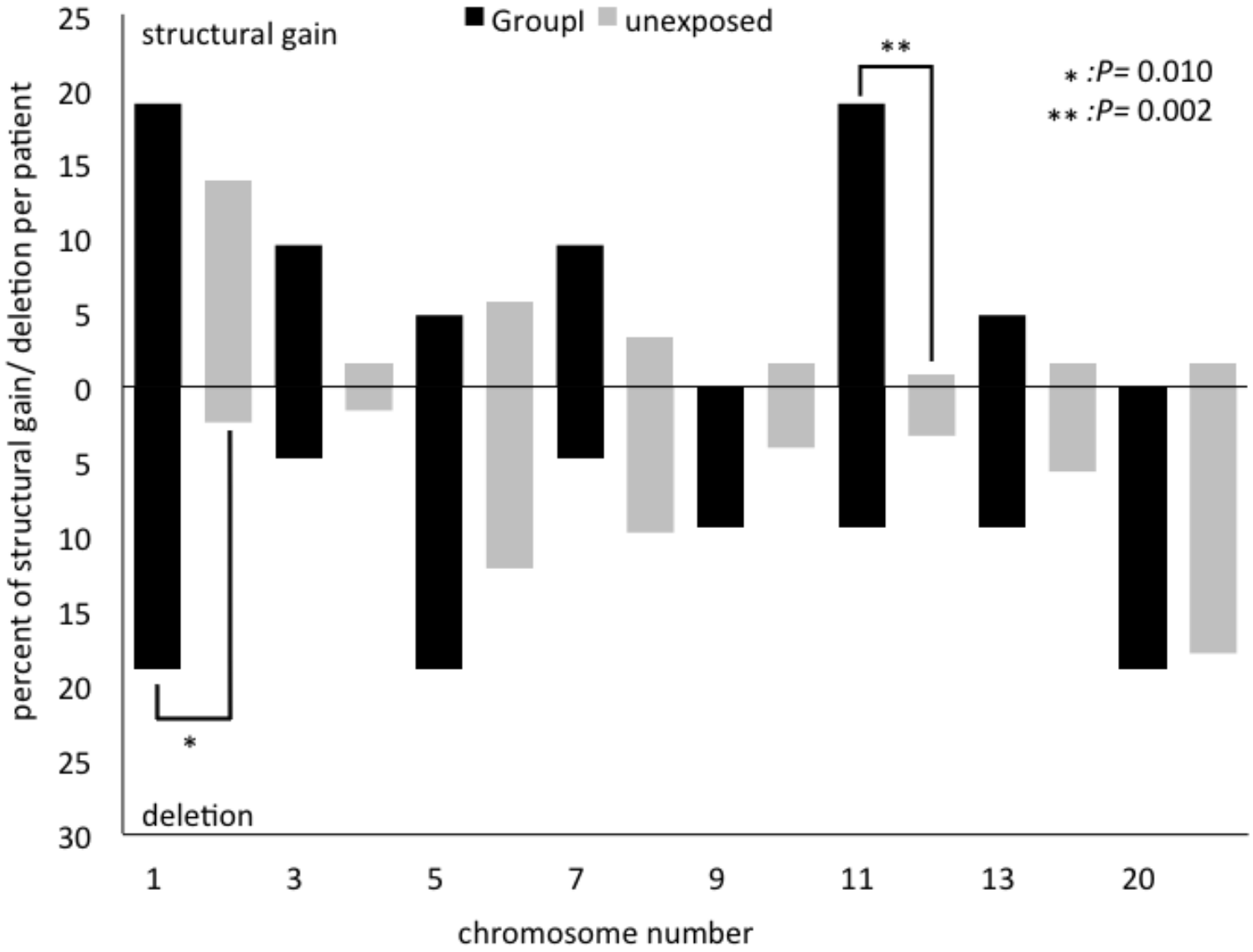

structural gain
Groupl
unexposed
** :P= 0.010
** :P= 0.002
percent of structural gain/ deletion per patient
25
20
15
10
5
0
5
10
15
20
25
30
*
**
deletion
1
3
5
7
9
11
13
20
chromosome number

Figure 2B

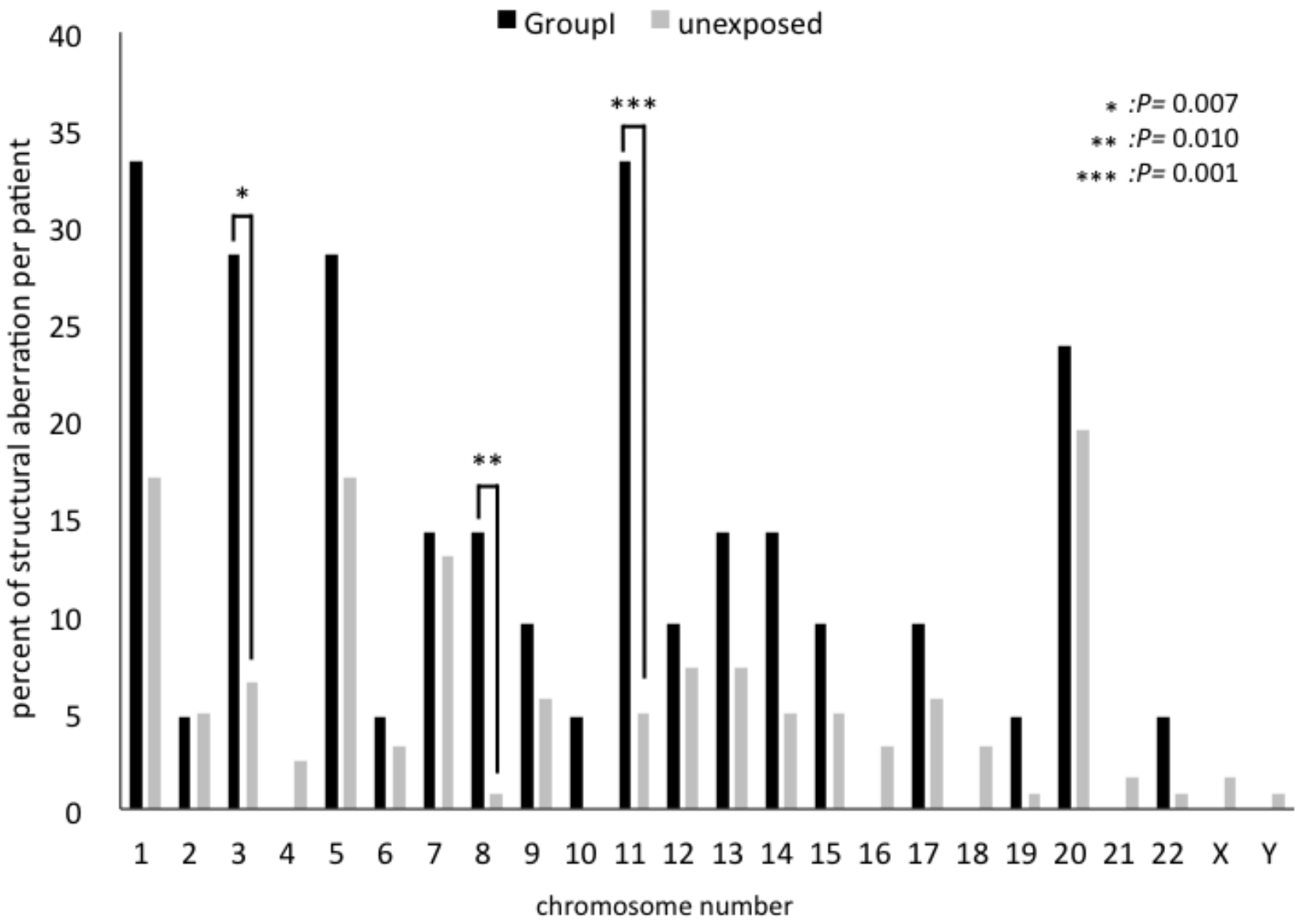

GroupI
unexposed
* :P= 0.007
** :P= 0.010
*** :P= 0.001
percent of structural aberration per patient
0
5
10
15
20
25
30
35
40
*
**
***
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X Y
chromosome number

Figure 3

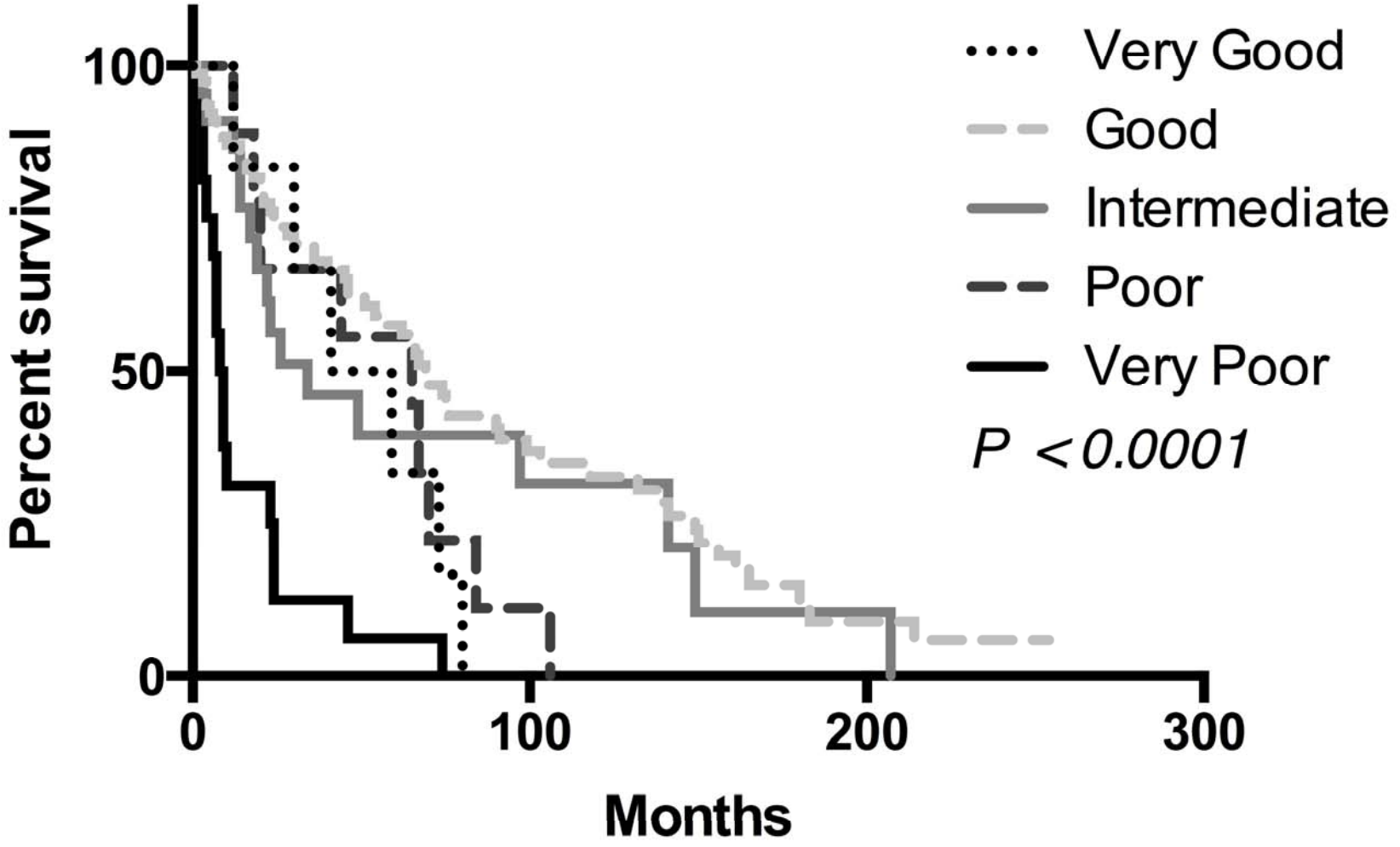

Very Good
Good
Intermediate
Poor
Very Poor
P <0.0001
Percent survival
100
50
0
0
100
200
300
Months

Figure 4A

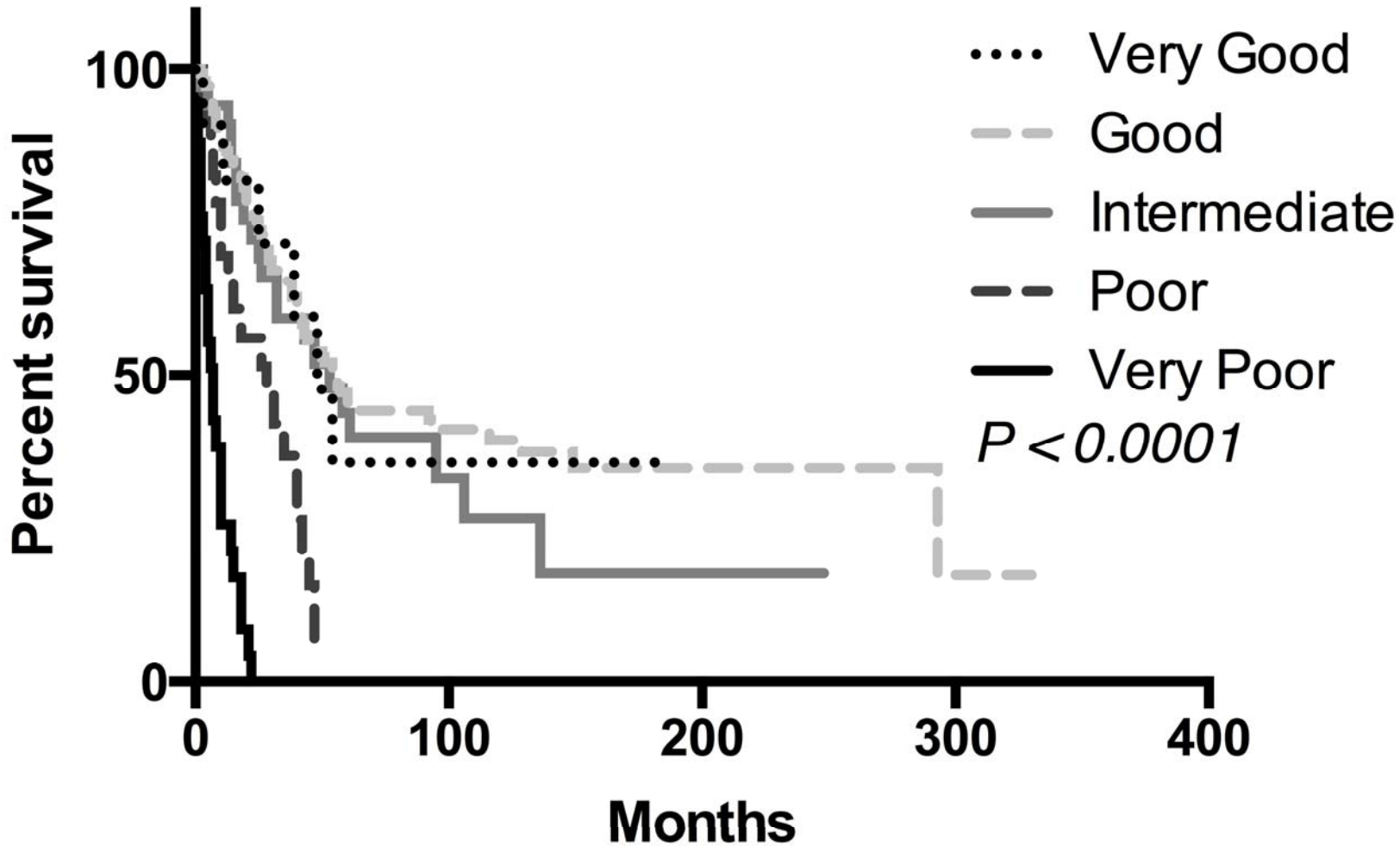

Percent survival
100
50
0
0
100
200
300
400
Months
Very Good
Good
Intermediate
Poor
Very Poor
P < 0.0001

Figure 4B

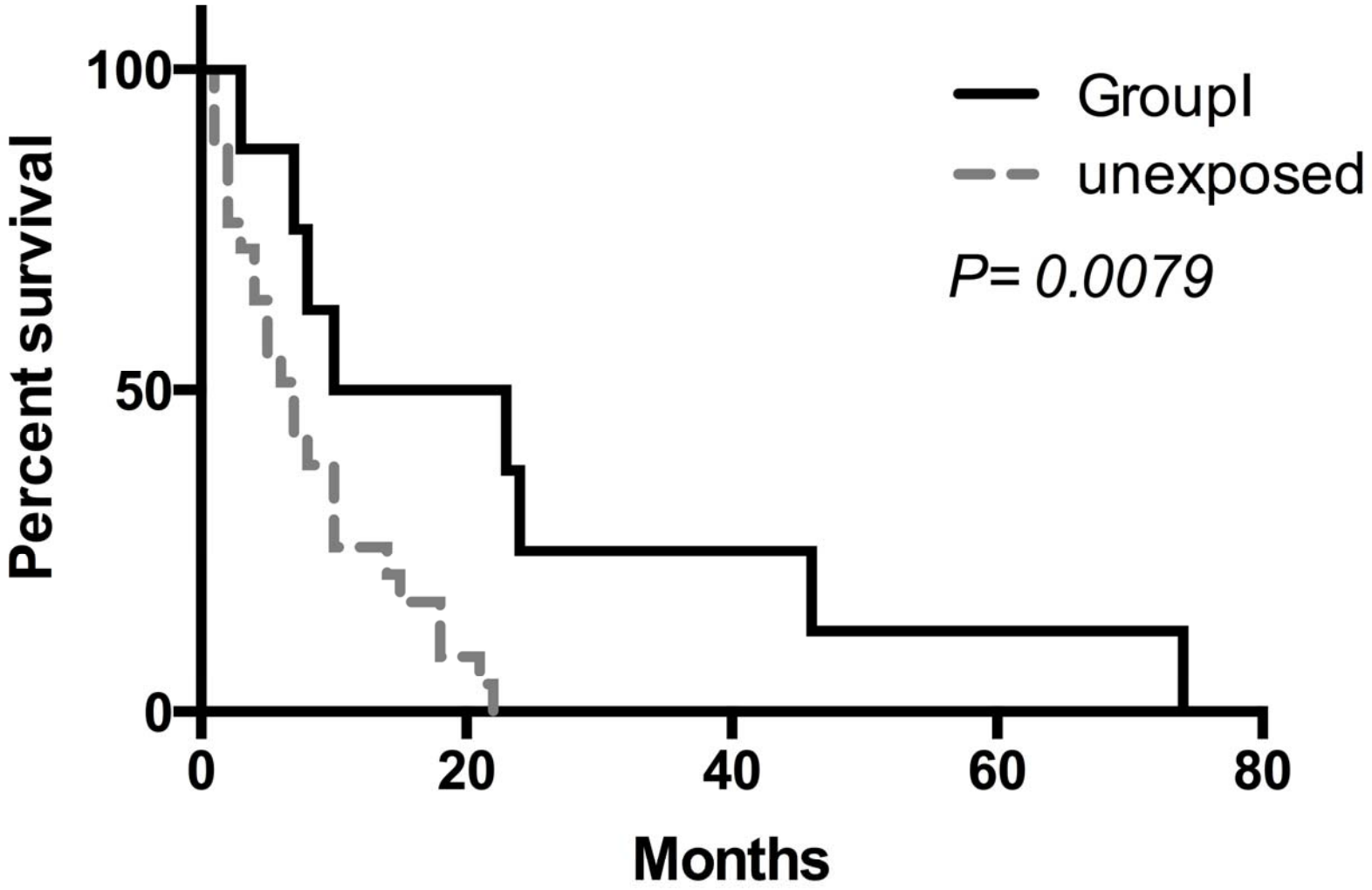

100
50
0
Percent survival
0
20
40
60
80
Months
GroupI
unexposed
P= 0.0079

Figure 5A

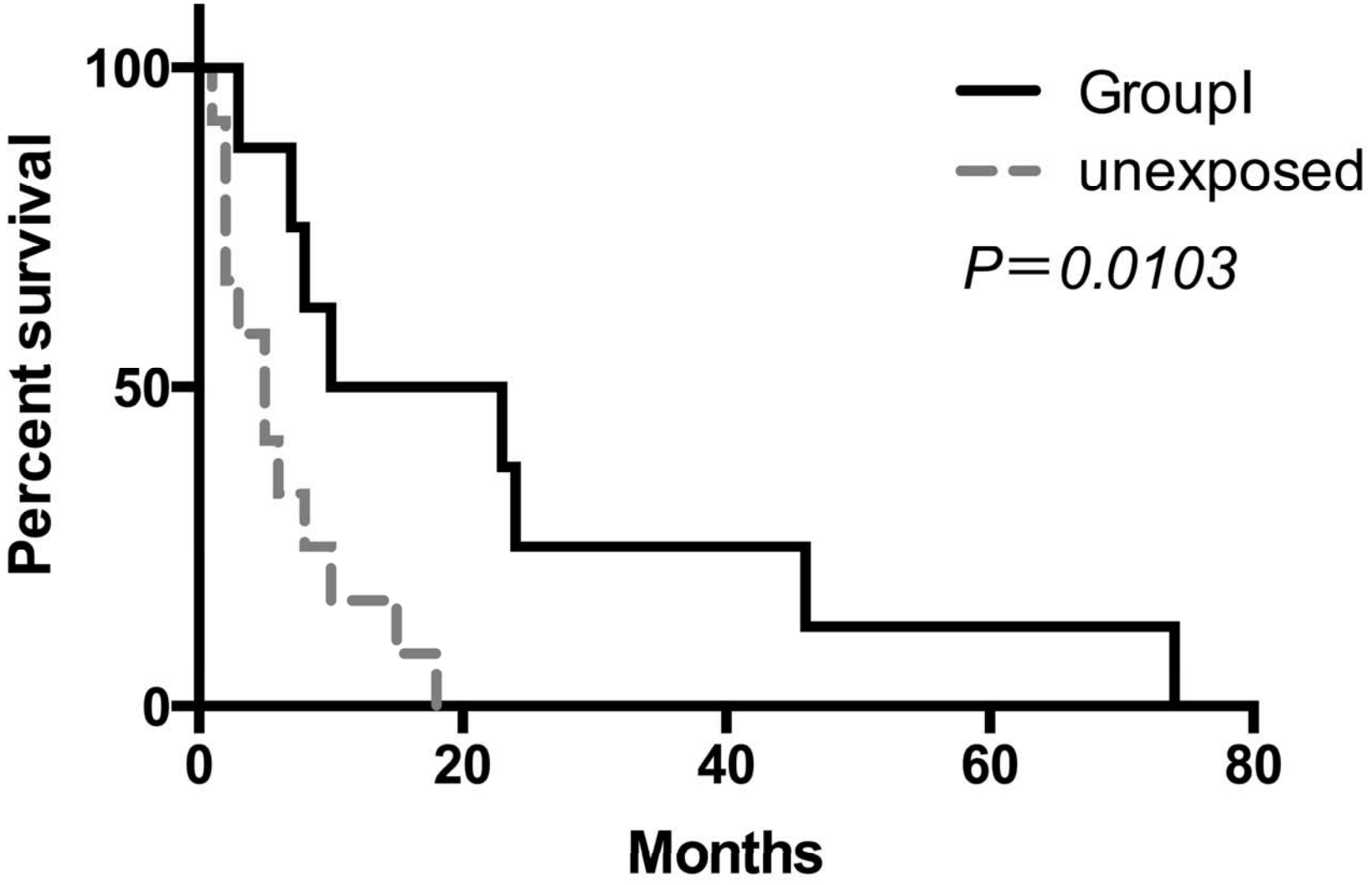
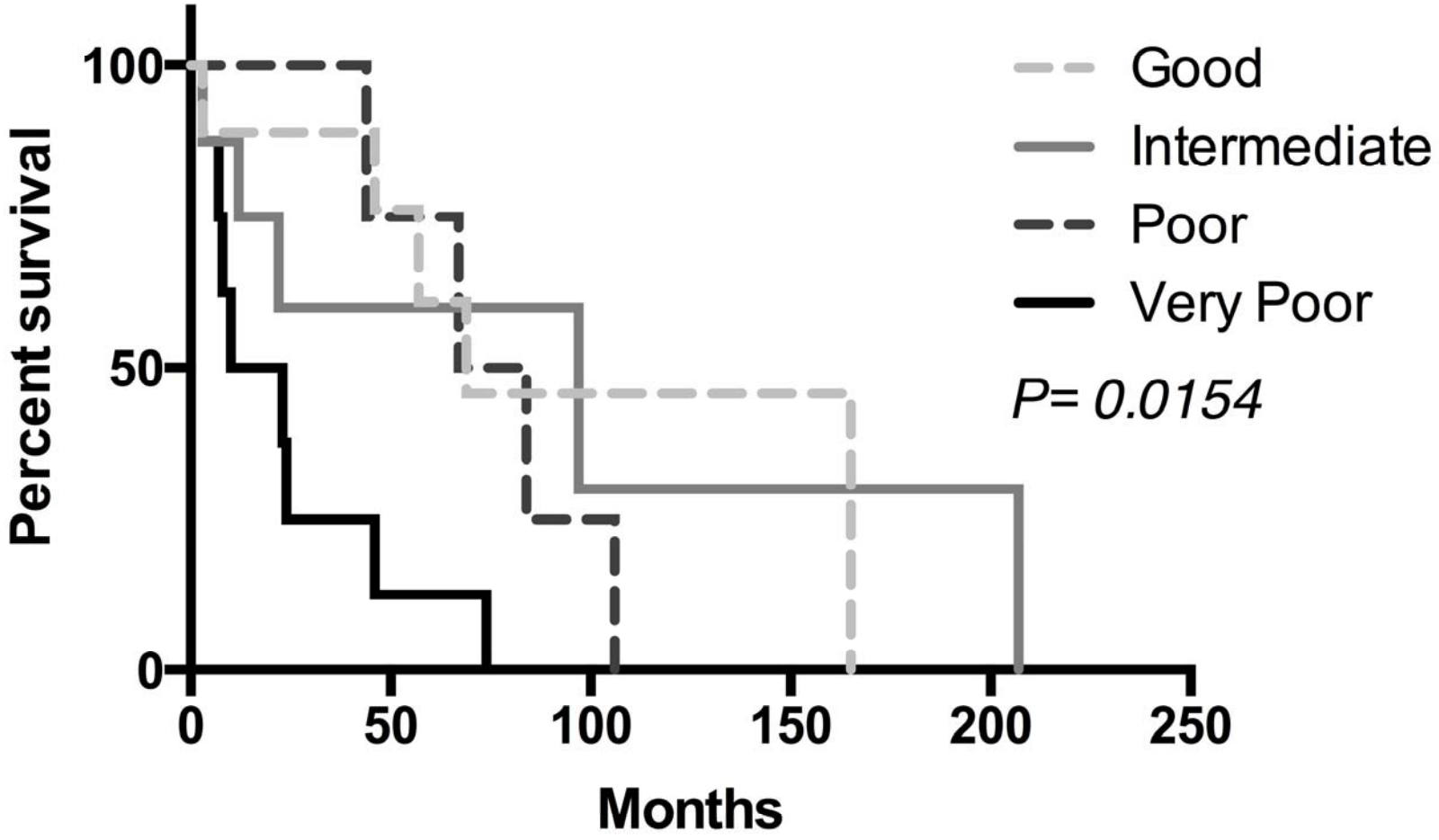
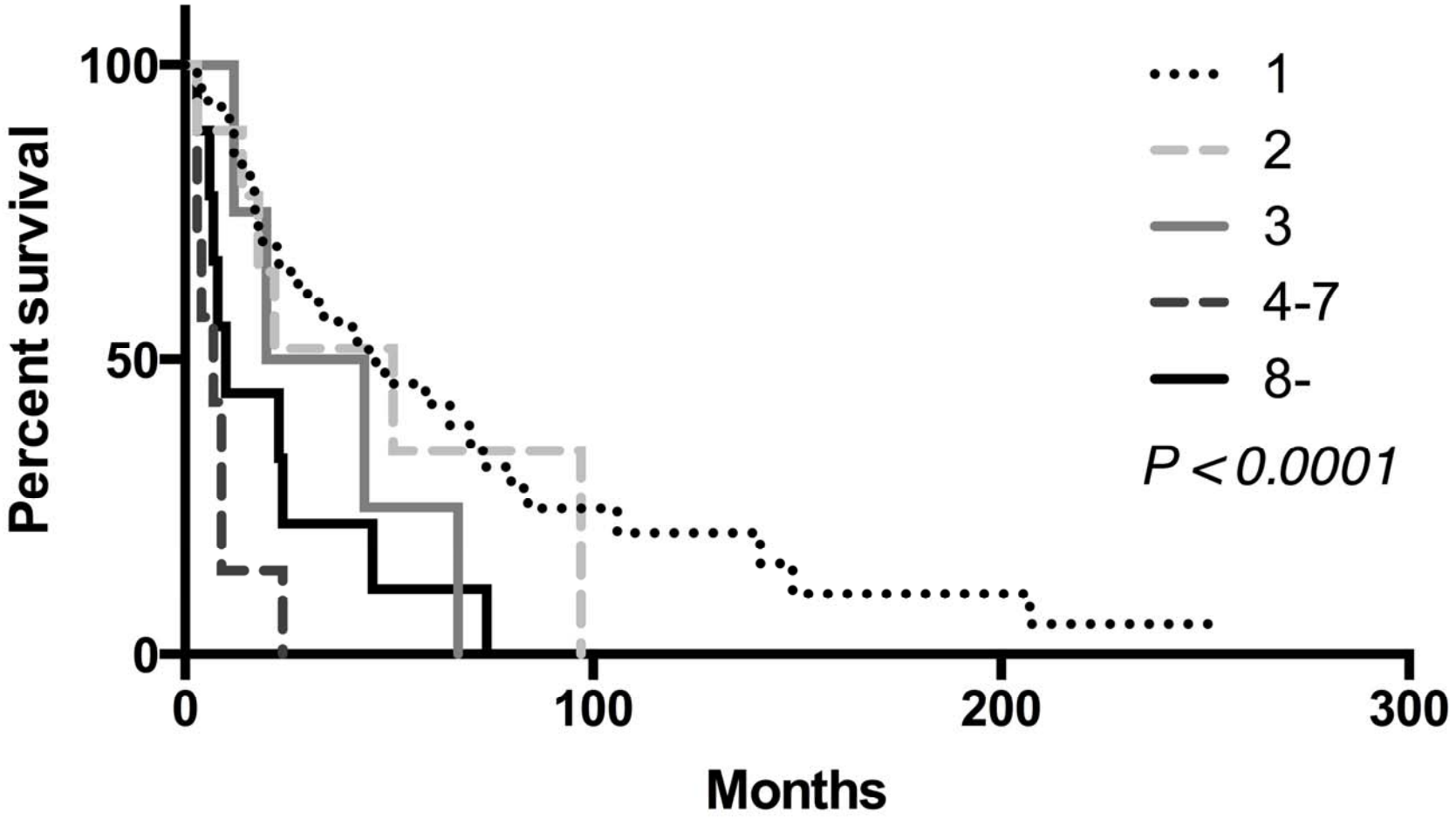

100
50
0
Percent survival
0
20
40
60
80
Months
GroupI
unexposed
P=0.0103

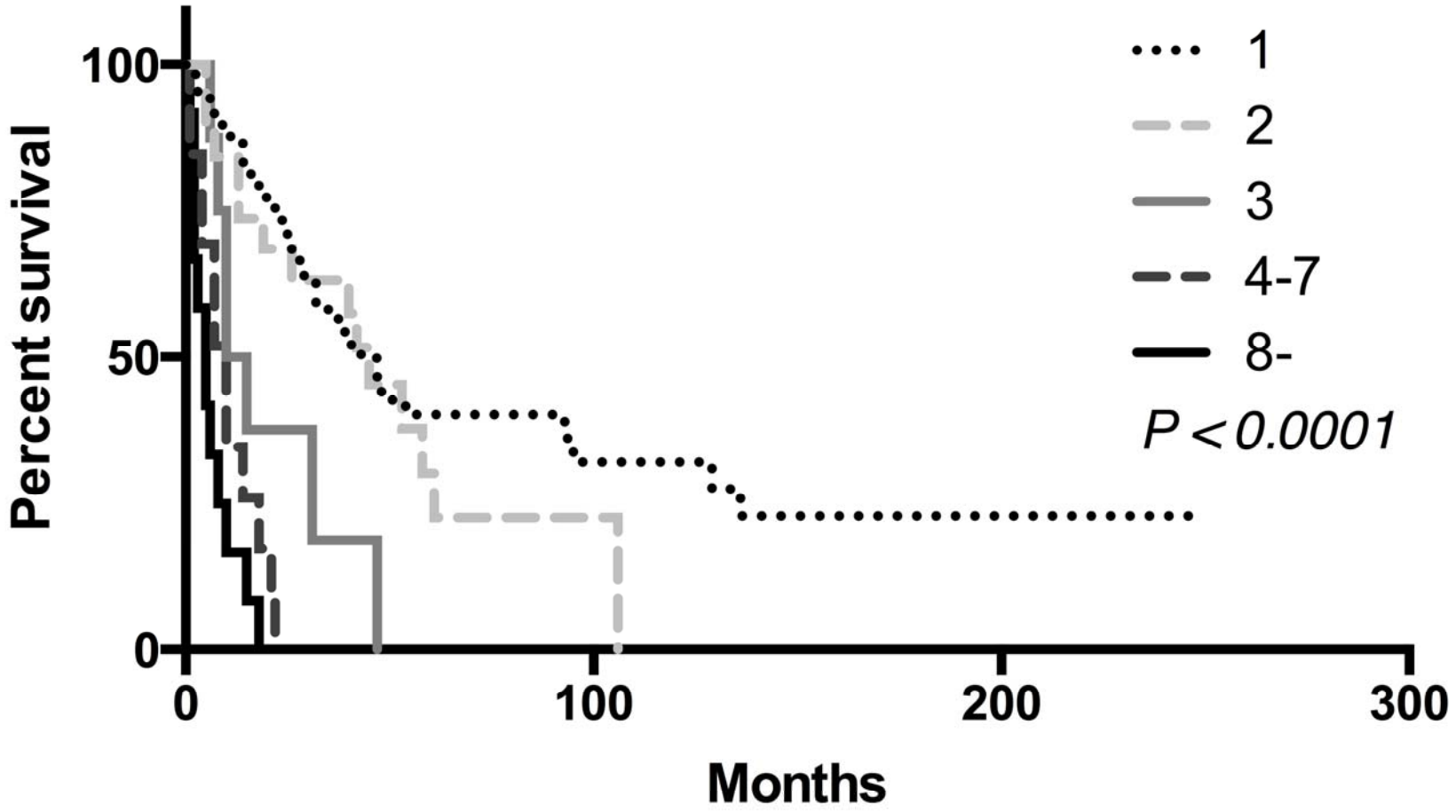
Figure 5B


Percent survival
100
50
0
0
50
100
150
200
250
Months
Good
Intermediate
Poor
Very Poor
P= 0.0154

Sup Figure 1



Sup Figure 2A



Sup Figure 2B