Would mean platelet volume/platelet count ratio be used as a novel formula to predict 22q11.2 deletion syndrome?

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Summary

Background: The diagnosis of 22q11.2 deletion syndrome depends on a time-consuming and expensive method, fluorescence in situ hybridisation (FISH).

Objectives: We aimed to determine new parameters which can aid for in the diagnosis of 22q11.2 deletion syndrome.

Methods: Twenty two patients with 22q11.2 or 10p13 deletion were evaluated retrospectively.

Results: Facial-dysmorphism and mental-motor retardation were detected in 100% of patients. Mean platelet (PLT) counts were lower (224,980 versus 354,000, \( p = 0.001 \)), mean PLT volume (MPV) (9.95 versus 7.07, \( p = 0.002 \)), and MPV/PLT \( \times 10^5 \) ratios (5.36 versus 2.08, \( p < 0.001 \)) were higher in patients with 22q11.2 deletion compared with the control group. Area under the receiver-operator characteristic (ROC) curve was 0.864, sensitivity was 84.6%, specificity was 90.1%, positive predictive value (PPV) was 91.7%, and negative predictive value (NPV) was 83.3% when MPV was 8.6. Area under ROC curve was 0.864, sensitivity was 76.9%, specificity was 90.1%, PPV was 90.1%, and NPV was 76.3% when PLT was 265,500. Area under ROC curve was 0.906, sensitivity was 84.6%, specificity was 100%, PPV was 100%, and NPV was 84.6% when MPV/PLT \( \times 10^5 \) was 3.3. Expression of PLT surface markers which were not in the GPIb-V-IX receptor complex (CD61, CD41a) increased as the surface area increased, but markers which were in a complex (CD42a, CD42b) did not change.

Conclusions: High MPV/PLT value can be a good predictor for the diagnosis of 22q11.2 deletion syndrome. We suggest that in patients with facial dysmorphism and retardation in neurodevelopmental milestones and if MPV≥8.6fl, MPV/PLT \( \times 10^5 \) ratio≥3.3 and PLT count ≤265,500/mm\(^3\), the patients should be tested by FISH analysis to confirm the 22q11.2 deletion. If there are no macrothrombocytes, the 10p13 deletion should be tested in suspected cases. (Asian Pac J Allergy Immunol 2016;34:166-73)

Keywords: 22q11.2 deletion syndrome, DiGeorge syndrome, mean platelet volume, platelet count, platelet membrane glycoproteins

Introduction

Many syndromes including DiGeorge syndrome (DGS), velo-cardiofacial syndrome, conotruncal anomaly face syndrome, and Opitz G/BBB syndrome have been found to share the same genetic deletion. All of these syndromes are now considered to comprise 22q11.2 deletion syndrome because of the heterozygote microdeletion in the 22q11.2 area. In some DGS phenotypes, a 10p deletion can be present instead of the 22q11.2 deletion. Therefore, as there is a patient with 10p13 deletion, we preferred to use the term ‘DGS’ to avoid confusion. 22q11.2 deletion syndrome is most common microdeletion syndrome, with an estimated prevalence of 1/3000-4000 live births. More than 35 genes are within the deletion region of
chromosome 22q11.2. One of the genes located within the typically deleted chromosomal region of > 90% of 22q11.2 patients is the glycoprotein (GP) Ibβ gene, which encodes for the beta polypeptide subunit of platelet GP Ib-V-IX. Therefore, affected patients could have macrothrombocytopenia.

The diagnosis of DGS depends on a method, fluorescence in situ hybridisation (FISH), which is time consuming and expensive but gives exact results. Therefore, we aimed to investigate whether platelet features which can be examined by easy and cheap methods could aid in predicting the diagnosis of DGS.

Methods

Twenty two patients admitted to Paediatric Allergy and Immunology department of Necmettin Erbakan University Meram Medical Faculty between April 2007 and February 2012 and with a diagnosis of DGS due to 22q11.2 or 10p13 deletion were evaluated retrospectively. The study was approved by the Institutional Review Board of our institution. The 22q11.2 deletion was shown using a standard probe (DiGeorge/VCFS TUPLE 1 or DiGeorge/VCFS N25) in 21 patients and the 10p13 deletion was shown by using the DiGeorge II (10p14) probe in 1 patient.

All total blood counts of patients obtained from electronic file records were evaluated for the presence of macrothrombocytes. Twenty children who were admitted to our department for routine control, who did not have infection at admission time and were age-matched with the study group were considered the control group. The instrument used for blood count was Cell-Dyn 3700 (Abbott, Lake Forest, IL, USA), using the optical channel of the instrument. Mean values of mean platelet volumes (MPV) and platelet counts in the previous blood counts were compared between the control and study groups. All other possible diseases causing macrothrombocytopenia (e.g., viral infections, liver disease, drug usage, immune thrombocytopenic purpura) in suspected cases were excluded. Platelet markers (CD41a FITC, CD42a FITC, CD42b PE, CD61FITC) that were examined to exclude the possibility of Bernard Soulier Syndrome (BSS) were evaluated. These flow cytometry analysis results were compared with the aged-matched values of our laboratory normal controls.

Statistical analysis was performed using the software SPSS 15.0 (Statistical Package For Social Sciences). Chi-square and Fisher's exact test were used to compare the difference between the frequency of the categorical data. Numeric data that complied with normal distribution were given as mean±standard deviation, whereas those that did not comply with normal distribution were given as the median (minimum-maximum). Mann-Whitney U test and Wilcoxon Rank sum test were used to compare the difference between the median of the data that did not comply with normal distribution. ROC analysis was used to calculate the most sensitive and specific values of MPV, PLT and MPV(fl)/PLT(×10⁵). P values below 0.05 were considered statistically significant in all analyses and the confidence interval (CI) was accepted as 95%.

Results

Twenty two patients (11 females, 11 males) were included in the study. Twenty one (95.5%) patients had a 22q11.2 deletion, and 1 (4.5%) had a 10p13 deletion. Median age at diagnosis was 38.5 months (1 month-35 years), and the median follow-up period was 14 (4-59) months. Two of the patients (9%) died; one died at 11 months of age because of pneumonia, the other died at 8 months of age because of multiorgan insufficiency and gastrointestinal haemorrhage induced after severe thrombocytopenia. The common features of the patients who died were the presence of conotruncal cardiac anomalies, the onset of complaints in the newborn period, hypoparathyroidism and severe lymphopenia.

All of the patients had facial dysmorphism and retardation in neurodevelopmental milestones. A history of recurrent respiratory tract infections was detected in 91%, velopharyngeal insufficiency in 77.2%, hypoparathyroidism, congenital cardiac disease and long-lasting infection resolved after parenteral antibiotic treatment in 54.5%, extremity anomaly in 50%, malnutrition in 31.8%, urogenital anomaly and pathological short stature (<-2.5 SD) in 27.2%, history of bone fracture and obesity in 18.1%, and hypothyroidism in 13.6% of the patients.

While low PLT counts and high MPV were noteworthy in the total blood count evaluations of the patients with 22q11.2 deletion, the values were normal in the patient with 10p13 deletion [mean PLT:247000/mm³(238000-256000/mm³) versus mean MPV:6.95fl (6.7-3.9fl)]. After this observation, all of the total blood counts of patients were re-evaluated in consideration of a common gene defect causing macrothrombocytopenia in the deletion area...
of 22q11.2 deleted patients. Twenty healthy children that admitted to department of paediatrics for routine control, who did not have infection at admission time and were age-matched with the study group were considered the control group. Mean values of MPVs and platelet counts in the previous blood counts were compared between the control and study groups.

All of the total blood counts thus far (totally 120 total blood count) were taken into consideration and mean values were obtained. Mean PLT was 224,980±18,700/mm³ in patients with a 22q11.2 deletion, 354,540±35,200/mm³ in the control group, and 247,000±22,300/mm³ in the patient with the 10p13 deletion (Figure 1). While mean PLT counts of the patients with 22q11.2 deletion were significantly lower when compared with the control group (p = 0.001) (Table 1), no significance was detected between the mean PLT of the patient with the 10p13 deletion and the control group (p = 0.16). When the PLT value was 265,500/mm³, sensitivity was found to be 76.9%, specificity 90.1%, positive predictive value 90.1% and negative predictive value 76.3% (Table 2). The area under receiver operating characteristic (ROC) curve for PLT count was found to be 0.881 [standard error (SE):0.069, 95% confidence interval (CI):0.745-1.017] (Figure 2).

Mean MPV was 9.95±0.46 fl in patients with a 22q11.2 deletion, 7.07±0.5 fl in the control group, (Table 1), 6.95±0.35fl in the patient with the 10p13 deletion (Figure 1). Mean MPV values were significantly higher in patients with a 22q11.2 deletion (p = 0.002), but were not different in the patient with the 10p13 deletion (p = 1). When mean MPV was 8.6, sensitivity was found to be 84.6%, specificity 90.9%, positive predictive value 91.7%, and negative predictive value 83.3% (Table 2). The area under the ROC curve for MPV was found to be 0.864 (SE:0.080, 95% CI:0.706-1.021) (Figure 2).

Ratio of MPV to PLT value was thought to be a guide in the diagnosis of DGS; therefore, the formula ‘MPV(fl)/PLT(/mm³)x10⁵’ was developed. Mean MPV/PLTx10⁵ was 5.36±0.79 in patients with a 22q11.2 deletion, 2.08±0.19 in control group (p = 0.000). There was not statistical difference between MPV/PLTx10⁵ values of 10p13 deleted patient and control group (p = 0.5). When MPV/PLTx10⁵ was 3.3, sensitivity was found to be 84.6%, specificity 100%, positive predictive value 100%, negative predictive value 84.6% (Table 2). The area under ROC curve for MPV/PLTx10⁵ was 0.906 (SE: 0.069, 95% CI: 0.771-1.041) (Figure 2); so, it can be speculated that MPV/PLTx10⁵ ratio is an important predictive parameter for the diagnosis of 22q11.2 deletion syndrome.
A novel formula to predict 22q11.2 deletion

Table 1. Comparison of mean MPV, PLT, MPV/PLT x 10^5 and PLT markers of the patients with 22q11.2 deletion and control group.

<table>
<thead>
<tr>
<th></th>
<th>22q11.2 del+ (min-max)</th>
<th>Control group (min-max)</th>
<th>P*</th>
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<tbody>
<tr>
<td>Mean MPV (fl)</td>
<td>9.95±0.46 (7-13)</td>
<td>7.07±0.5 (5.18-8.4)</td>
<td>0.002</td>
</tr>
<tr>
<td>Mean PLT / (mm³)</td>
<td>224.98±18.700 (10.000-375.000)</td>
<td>354.000±35.240 (249.000-663.000)</td>
<td>0.001</td>
</tr>
<tr>
<td>Mean MPV/PLT x 10^5</td>
<td>5.36±0.79 (3.3-12)</td>
<td>2.08±0.19 (1.1-3.2)</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>CD41a MFI</td>
<td>107.4±23.7 (55-271)</td>
<td>77.7±13.3 (32-150)</td>
<td>0.43</td>
</tr>
<tr>
<td>CD41a%</td>
<td>77.9±7.2 (88.1±2.4)</td>
<td>81.5±3.6 (212-443)</td>
<td>0.69</td>
</tr>
<tr>
<td>CD42b MFI</td>
<td>304.4±50.8 (115-724)</td>
<td>310.5±23.9 (212-443)</td>
<td>0.51</td>
</tr>
<tr>
<td>CD42b%</td>
<td>88.3±4.2 (97.1±0.37)</td>
<td>97.1±0.37 (77-230)</td>
<td>0.01</td>
</tr>
<tr>
<td>CD61 MFI</td>
<td>202.5±22.1 (78-315)</td>
<td>133±13.1 (77-230)</td>
<td>0.028</td>
</tr>
<tr>
<td>CD61%</td>
<td>88.4±4.3 (97.3±0.3)</td>
<td>97.2±0.3 (32-166)</td>
<td>0.06</td>
</tr>
<tr>
<td>Mean CD42a MFI</td>
<td>75±11.2 (27-271)</td>
<td>91.9±6 (32-166)</td>
<td>0.088</td>
</tr>
<tr>
<td>CD42a%</td>
<td>84.4±4.7 (96.7±0.74)</td>
<td>96.7±0.74 (77-230)</td>
<td>0.002</td>
</tr>
<tr>
<td>Mean CD42b MFI/</td>
<td>1.75±0.37 (0.54-4.6)</td>
<td>2.52±0.31 (1.61-4.84)</td>
<td>0.019</td>
</tr>
<tr>
<td>CD61 MFI</td>
<td>4.97±1.38 (0.63-13.5)</td>
<td>5.24±0.94 (1.46-11.6)</td>
<td>0.43</td>
</tr>
</tbody>
</table>

*: There was not statistical difference between PLT, MPV, MPV/PLT x 10^5 values of 10p13 deleted patient and control group (p: 0.16, 1, 0.5, respectively).

The percentages and mean fluorescence intensities (MFI) of PLT markers (CD61, CD41a, CD42a, CD42b) and CD42b MFI/CD41a MFI ratio of 12 patients with 22q11.2 deletion that had been examined to exclude the platelet surface marker pathologies because of existence of macrothrombocytopenia were compared with the age-matched laboratory normal controls. These parameters were also studied in the patient with the 10p13 deletion. When these values were compared with the control group, it was found that mean CD61 MFI was higher (p = 0.028), CD42b%, CD42a% and CD42b/CD61 ratio were lower (p: 0.01, 0.002, 0.019, respectively), and mean CD41a MFI, CD42a MFI, CD42b MFI, CD61%, CD41a%, CD42b/CD41a were not different (p: 0.43, 0.08, 0.51, 0.065, 0.69, 0.43, respectively) in the patients with a 22q11.2 deletion (Table 1, Figure 3). No statistical difference was detected between the PLT surface markers of the patient with the 10p13 deletion and the control group.

As there was no appropriate kit for flow cytometry, we could not study CD42c, which recognises GP1b beta, and CD42d, which recognises GPV. CD42b is a monoclonal antibody which is used to detect GP1b alpha, CD42a to detect GP IX and GPp1b-V-IX complex. CD61 antigen is also known as GPIIIa and is deficient in patients with Glanzmann’s thrombasthenia. The CD41a molecule, which is also known as GPIIia and is deficient in patients with Glanzmann’s thrombasthenia. The CD41a molecule, which is also known as GPIIib, non-covalently binds to CD61 (GPIIIa) and composes the CD41/CD61 complex which plays a role in PLT aggregation. Normally, it is expected that while the surface area of a platelet increases, expression of the surface markers also increases. This knowledge is linked to our results; we found that CD61 MFI increased.

Figure 2. ROC curves for values of MPV, PLT and MPV/PLT x 10^5 in patients with 22q11.2 deletion. The area under ROC curve for PLT count was 0.881 (SE:0.069, 95%CI:0.745-1.017), for MPV 0.864 (SE:0.080, 95% CI:0.706-1.021), and for MPV/PLT x 10^5 0.906 (SE:0.069,%95 CI:0.771-1.041).
Table 2. Diagnostic performance of PLT, MPV and MPV(fl)/PLT (/mm$^3$)$\times 10^5$ ratio in patients with DiGeorge syndrome

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLT (&lt;266,000/mm$^3$)</td>
<td>90.9</td>
<td>76.9</td>
<td>79.6</td>
<td>90.9</td>
</tr>
<tr>
<td>MPV (&lt;8.62 fl)</td>
<td>84.6</td>
<td>90.9</td>
<td>91.7</td>
<td>83.3</td>
</tr>
<tr>
<td>MPV(fl)/PLT (/mm$^3$)$\times 10^5$ (&gt;3.3)</td>
<td>84.6</td>
<td>100</td>
<td>100</td>
<td>84.6</td>
</tr>
</tbody>
</table>

PPV: Positive predictive value, NPV: Negative predictive value

significantly ($p = 0.028$), and CD41a MFI increased mildly (but not statistically) ($p = 0.43$). However, contrary to expectations, CD42b MFI, which falls within the GP1b-V-IX complex, did not change ($p = 0.51$), CD42b percentage decreased ($p = 0.01$), CD42a MFI decreased mildly (but not statistically) ($p = 0.088$), and CD42a percentage ($p = 0.002$) and CD42b/CD61 ratio decreased significantly ($p = 0.019$) in patients with a 22q11.2 deletion as the surface area of the platelet increased.

When the patients were questioned in terms of bleeding diathesis, it was found that 10 (45.5%) of the patients with a 22q11.2 deletion had a history of bruising easily, 2 (9%) had recurrent epistaxis, 2 (9%) had prolonged bleeding after cuts, and 2 (9%) had a haemorrhage in the gastrointestinal system. None of these findings were seen in the patient with the 10p13 deletion or the control group.

Discussion

Age at diagnosis and the number of patients with DGS depends on the experience and awareness of the related clinicians and severity of phenotype. DiGeorge syndrome is not known adequately among clinicians, and it is believed wrongly that all of the characteristic features of DGS should coexist. Even when DGS is considered as a diagnosis in a patient, FISH analysis is needed to confirm the diagnosis, but this is an expensive and hardly attainable method. Based on this, we aimed to investigate new parameters for the diagnosis of DGS by using total blood count, which is a cheap and easily attainable method.

Haematological pathologies like thrombocytopenia, pancytopenia and anaemia, especially together with autoimmunity, can be seen in the patients with DGS.$^{3,4}$ Although the most common reported haematological disorder is immune thrombocytopenia, cases of non-autoimmune thrombocytopenia are also reported.$^{5}$ Ozbek et al. reported high myelodysplasia score and

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**Figure 3.** Comparison of mean CD42b MFI/CD61 MFI ratios (A) and CD61 MFI values (B) between groups. Mean CD42b/CD61 ratio were lower (0.019), mean CD61 MFI was higher ($p=0.028$) in the patients with 22q11.2 deletion when compared with the control group. The figures of CD41a MFI, CD41a%, CD42b MFI, CD42b%, CD61%, CD42a MFI and CD42b MFI/CD41a MFI were not shown here.
A novel formula to predict 22q11.2 deletion

High macropolycytes (hypersegmented neutrophils larger than normal neutrophils) in 5 patients with a 22q11.2 deletion, similar to that in patients with bacterial infection. This also supports the theory that genetic defects can cause abnormal haematological findings as well as characteristic findings of DGS.6

One of the genes in typical deletion area is the GPIb beta gene, which encodes the beta polypeptide subunit of PLT GPIb-V-IX. GPIb-V-IX is a primary adhesion receptor for von Willebrand factor and is critical for PLT adhesion and homeostasis.7 Optimal GPIb-V-IX function and expression on the PLT membrane surface depends on at least three of its four subunits - GPIbα, GPIbβ and GPIX.7 GPIb is a heterodimer of GPIb alpha and GPIb beta, which together with GPIX and GPV, act as the PLT receptor for von Willebrand factor. A quantitative or qualitative defect in GPIb-V-IX, caused by mutations in any one of the GPIb alpha, Ib beta and GPIX genes, results in a rare but severe bleeding disorder called Bernard-Soulier Syndrome (BSS), which is inherited as an autosomal recessive condition.8

BSS is characterised by macrothrombocytopenia, prolonged bleeding time, impaired PLT agglutination response to ristocetin and low dose thrombin in vitro. In most cases, BSS patients also have decreased expression of the GPIb-V-IX receptor complex on PLT membrane surface.8

While more than 90% of patients with DGS have either 1.5 or 3 Mb microdeletion on chromosome 22q11.2 containing the GPIb beta gene; they are obligatory heterozygotes for GPIb beta deficiency, similar to the heterozygous parents of BSS patients.9 Heterozygote carriers for BSS also do not have bleeding diathesis but they have giant PLTs.10 The possibility of having BSS is greater in DGS patients compared to normal individuals.11,12 However, there are few reports which show that DGS patients have an increased bleeding tendency.9,13 Although macrothrombocytopenia has been noted in some DGS patients, it was not considered a classical manifestation of DGS. Van Geet et al. studied a family of 3 DGS patients who were GPIb beta heterozygotes, and showed that PLT aggregation in response to ristocetin, a GPIb-V-IX dependent agonist, was normal, but found reduced PLT survival time.14 Conversely, Pallotta et al. showed reduced PLT aggregation response to ristocetin in a patient who was a GPIb beta heterozygote.9

Consistent with our study, Liang et al. and Naqvi et al. reported low PLT counts and high MPV values compared with the control group.13,15 (Table 3). So far, there have been no reports containing predictive values about PLT counts in DGS. In line with our study, Naqvi et al. reported that MPV <8.5 excludes 22q11.2 deletion (sensitivity: 100%), and if MPV >10 (specificity: 90%), the patient most likely had deletion.15 In our study, PLT counts of patient and control groups were higher, while MPV values were lower than the other studies. To determine whether the cause of this is due to methods used in the laboratory or social differences, further research is needed. Lawrence et al. found PLT counts of DGS patients to be 70% of control group,16 while we found this ratio to be 63%.

Although it was denoted in previous studies that there was a negative correlation between PLT and MPV values in 22q11.2 deleted patients, there are very few studies that deal with a practical method that could be used in diagnosis.14,17 Our results show that macrothrombocytopenia is a very good predictive parameter in the diagnosis of DGS. In probable cases, if MPV≥8.6fl (sensitivity 84.6%, specificity 90.9%), MPV/PLT×105 ratio ≥3.3

<table>
<thead>
<tr>
<th>Authors/publication year</th>
<th>Mean MPV (fl) in patients (range)</th>
<th>Mean MPV (fl) in controls (range)</th>
<th>P</th>
<th>Mean PLT in patients (range)</th>
<th>Mean PLT in controls (range)</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>Liang HP, 2007</td>
<td>10.6 (9.4-12.6)</td>
<td>8.3 (7.3-9.6)</td>
<td>P &lt; 0.001</td>
<td>175.000/mm² (113.000-300.000)</td>
<td>289.500/mm² (221.000-362.000)</td>
<td>P &lt; 0.001</td>
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<td>Naqvi N, 2011</td>
<td>10.9 (8-15.5)</td>
<td>8.6 (6.6-13.2)</td>
<td>P &lt; 0.001</td>
<td>155.000/mm² (100.000-350.000)</td>
<td>225.000/mm² (105.000-514.000)</td>
<td>P &lt; 0.001</td>
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<tr>
<td>Gokturk B, 2016</td>
<td>9.95±0.46 (5.5-13)</td>
<td>7.07±0.5 (6.5-7.6)</td>
<td>P = 0.002</td>
<td>224.980±18.700/mm² (10.000-675.000)</td>
<td>354.540±35.200/mm² (319.000-389.000)</td>
<td>P = 0.001</td>
</tr>
</tbody>
</table>

Table 3. Mean MPV and PLT values of patients with 22q11.2 deletion reported in literature
(sensitivity 84.6%, specificity 100%) and PLT count ≤265,500/mm² (sensitivity 76.9%, specificity 90.1), we can say that the patient probably has a 22q11.2 deletion. We believe that the formula, MPV/PLT×10⁵, which is used for the first time, could contribute to the literature.

To date, two reports exist that have studied platelet surface markers using flow cytometry device.⁹,¹³ Pallotta et al. evaluated a 17-year-old patient with a 22q11.2 deletion and macrothrombocytopenia, and found that GP Ib alpha (CD42b) expression was half with respect to the control platelets.⁹ Liang et al. studied 21 patients with 22q11.2 deletion, and found 50% reduction in PLT GPIb:GPIIb (CD42b/CD41a) surface expression ratio with respect to platelets from their unaffected parents. They also mentioned that expression of GPIb-IIIa (CD41a-CD61) receptors increased on PLT surface as the PLT size increased; conversely, GPIb-V-IX antibody MFI (CD42a) remained similar to their unaffected parents.¹³ In both studies, it was shown that patients with a 22q11.2 deletion were also heterozygotes for the GPIb beta gene; consequently, they mentioned that maturation and surface expression of the GPIb-V-IX receptor complex were insufficient. Similar to the study of Liang et al., we found PLT surface markers which were not a component of the GPIb-V-IX complex to be higher than the control. Based on these findings, we concluded that the expression of PLT surface markers which were not in the GPIb-V-IX receptor complex increased as the surface area increased, but markers which were in the GPIb-V-IX receptor complex could not increase or decreased.

There is no clear consensus about which patient should be chosen to test for DGS. Some units suggest investigating patients who have typical phenotypic features plus conotruncal cardiac anomaly, and some newborns with congenital cardiac disease.¹⁸ However, these suggestions are not always cost-effective and attainable. Using MPV and MPV/PLT×10⁵ formula before application of FISH analysis, especially in patients with congenital cardiac disease, seems to be a usable method to choose the patient for testing. If DGS is suspected and MPV is normal, a 10p13 deletion should be looked for instead of a 22q11.2 deletion. Thus, the cost of FISH analysis can be halved by using only one probe instead of two.

There are limitations to our study. First, although almost half of our patients had a positive history of possible bleeding tendency, we did not study PLT function tests, and we chose the control group among those without a bleeding tendency. Therefore, we cannot comment on PLT functions of DGS patients. Second, we do not really know whether our patients are heterozygous for the GPIb beta gene or not. Another objection can be the limited number of our patients. A multicentre prospective study including a larger patient and control group should confirm our results and support the new formula.

Conclusions
Every patient with facial dysmorphism and retardation in developmental milestones should be tested for DGS. For suspicious cases, MPV and PLT values can aid in diagnosis. We suggest that if MPV≥8.6fl, MPV/PLT×10⁵ ratio ≥3.3 and PLT count ≤265,500/mm², the patient should be tested by FISH analysis to confirm a 22q11.2 deletion. If a patient with manifestations of DGS does not have macrothrombocytes, 10p13 deletion should be tested for.

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Conflict of interest
None declared.

References
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