Leukocyte Activation in Obese Patients

Effect of Bariatric Surgery

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Abstract: The rising prevalence of obesity is a major global health problem. In severe obesity, bariatric surgery (BS) allows to obtain a significant weight loss and comorbidities improvement, among them one of the factors is the thrombotic risk. In this observational study, we measured indices of leukocyte activation in severely obese patients as markers of increased thrombotic risk in relation with serum markers of inflammation before and after BS.

Frequency of polymorphonuclear neutrophil-platelet (PLT) and monocyte (MONO)-PLT aggregates as well as of tissue factor (TF) expressing MONOs was measured in the peripheral blood of 58 consecutive obese patients and 30 healthy controls. In 31 of the 58 obese patients, data obtained at the enrollment were compared with those obtained at 3, 6, and 12 months after BS.

Compared with healthy controls, obese patients showed a higher frequency of polymorphonuclear leukocyte (PMNL)-PLT aggregates (12.31 [3.45–4.56]% vs 2.64 [7.31–8.97]%, P = 0.001), MONO-PLT aggregates (12.31 [7.33 [10.38–14.24]% vs 8.14 ± 2.22 [7.31–8.97]%, P < 0.001), and TF expressing MONOs (4.01 ± 2.11 [3.45–4.56]% vs 2.64 ± 1.65 [2.02–3.25]%, P = 0.002). PMNL-PLT and MONO-PLT aggregate frequency was positively correlated with TF expressing MONOs (R² = 0.260, P = 0.049 and R² = 0.318, P = 0.015, respectively).

BS was performed in 31 patients and induced a significant reduction of the body mass index, and waist and hip circumferences. These effects were associated with a significant decrease of PMNL-PLT aggregates at 12 months (7.58 ± 2.27 [6.75–8.42]% vs 4.47 ± 1.11 [3.93–5.01]%, P < 0.001), and a reduction of TF expressing MONOs at 6 (3.82 ± 2.04 [3.07–4.57]% vs 1.60 ± 1.69 [0.30–2.90]%, P = 0.008) and 12 months (3.82 ± 2.04 [3.07–4.57]% vs 1.71 ± 0.54 [1.45–1.97]%, P = 0.001) after BS.

These data suggest that leukocyte-PLT aggregate formation and MONO activation represent an important mechanism underlying the increased thrombotic risk of obese patients. We also show that BS is effective in normalizing these inflammatory indices.

INTRODUCTION

Obesity is a major independent risk factor for cardiovascular disease (CVD) and represents a critical health issue worldwide. Several mechanisms link obesity to CVD. Adipose tissue is an active endocrine and paracrine organ which releases a large number of cytokines and bioactive mediators, which influence body weight homeostasis as well as a number of metabolic and coagulative parameters.1–7 Adipose tissue-released soluble factors have proinflammatory effects, thereby explaining the chronic systemic inflammation and abnormal activation of coagulation cascade that contribute to the thrombotic tendency in these patients.8,9

In recent years, microRNAs have emerged as ubiquitous regulators of pathophysiological and cellular processes in obesity-related conditions9–11 such as CVD.11 miRNAs dysregulation is involved in the development of obesity-related CVD, thus indicating potential for novel therapeutic strategies in the management of obesity.15

Further supporting the relationship between obesity and CVD, a positive association between a high body mass index (BMI) and a number of coronary heart disease risk factors has been reported in a recent large cohort study in patients hospitalized with myocardial infarction.14 In this study, obesity was found strongly and positively associated with at least 3 of the 5 risk factors studied (hypertension, dyslipidemia, and diabetes).10

The first clinical approach to obese patients is a nonsurgical program with a dietary regimen, appropriate exercise, and behavioral modification and support. Bariatric surgery (BS) is recommended if patients fail to obtain a substantial and sustained weight reduction with nonsurgical procedures, as it is often the case when the BMI is >35 kg/m². BS has been shown to produce a durable decrease in excess body weight and improve associated comorbidities, namely diabetes, dyslipidemia, hypertension, and cardiovascular dysfunction.16–21 The consequent vascular risk factors reduction is the main factor responsible for the prognostic improvement of obese patients after BS.22,23

In the last years, increased levels of circulating leukocyte-platelet (PLT) aggregates have been observed in a range of inflammatory, metabolic, and CV diseases associated with...
increased vascular risk. In general, leukocyte-PLT interactions play a pivotal role in promoting the inflammatory reactions of the vessel wall that are fundamental for the initiation and progression of atherothrombosis. In addition, PLT are fundamental partners of the immune system and modulate the inflammatory immune response, most frequently by amplification. These data indicate that activation of white blood cells occurs in obesity and that BS might be regarded as an efficient approach to blunt this activation. Supporting this view, recent data show that BS reduced plasma level of certain inflammatory cytokines, in particular interleukin (IL)-8 and IL-1β, which are released by activated leukocytes.

A growing body of evidence assigns to the tissue factor (TF) a central role in thrombosis and inflammation-associated atherosclerosis. TF is the primary cellular initiator of the blood coagulation cascade. It is also expressed on the cell membrane of circulating monocytes (MONOs), and overexpression of this protein is an indicator of cell-mediated thrombogenicity in patients with CVD.

CRP is a hepatic acute-phase protein that increases following secretion of certain cytokines, that is IL-6 and tumor necrosis factor-α (TNF-α) by macrophages and adipocytes. Several studies have shown that CRP is a marker of increased atherothrombotic risk and can therefore be taken as an independent predictor of cardiovascular events. In obese patients, CRP is directly related to BMI and visceral fat accumulation, and concurs to thrombosis, instability and rupture of atherosclerotic plaque. CRP is also directly associated with MONO-PLT aggregate frequency and seems to promote TF synthesis in MONOs.

On this background, we set about investigating the frequency of polymorphonuclear leukocyte (PMNL)-PLT and MONO-PLT aggregates, and the intensity of TF expression by MONO, to verify whether these parameters can be dependent on the proinflammatory and prothrombotic status observed in obese patients. The effect of BS on these parameters in relationship with BMI, CRP, and other hematological parameters modulation was also studied.

METHODS

Patients and Study Protocol

The study was designed as a case-control study and a longitudinal study following BS. Fifty-eight (33/25 F/M; mean age 42.8 ± 10.9 [40.0–45.7] years) severely obese (BMI > 40 kg/m² or BMI between 35 and 40 kg/m² and serious coexisting medical conditions) outpatients followed by Catholic University Obesity Center were consecutively enrolled in the study between September 2011 and March 2014. We also studied 30 healthy volunteers (BMI < 25 kg/m²) recruited from hospital and laboratory personnel, appropriately matched for sex and age.

In the longitudinal section of our study, 31 patients underwent laparoscopic Roux-é-Y gastric bypass (RYGB). These patients were evaluated preoperatively at baseline (T0) and at 3 (T1), 6 (T2), and 12 (T3) months after BS. At each visit, vital signs, weight, BMI, and waist and hip circumferences were recorded. Blood was obtained from an antecubital vein through a 21-gauge butterfly needle with a large tourniquet and was collected at each visit early in the morning after an overnight fasting, and before any drug administration. Blood cell count, plasma glucose, glycated hemoglobin (HbA1c), total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides, and CRP were measured.

Informed consent was obtained in accordance with the Declaration of Helsinki. The study was approved by the Ethical Committee of the Catholic University of the Sacred Heart in Rome, Italy.

PMNL-PLT and MONO-PLT Aggregates

Blood samples were collected into sterile siliconized tubes containing trisodium citrate (0.129 mol/L, 1:10 vol/vol) and processed within 15 minutes after collection, as follows. Blood was immediately fixed in 0.5% paraformaldehyde to avoid artefactual cellular activation and aggregate formation. Aliquots of 100 μL of fixed blood were incubated for 10 minutes in the dark with saturating concentrations of fluorochrome-conjugated mouse monoclonal antibodies (Moabs) specific to PMNL and MONO (CD11b), PLT (CD41a), and activated PLTs (CD62P). TF expression by MONO was assessed using the Moab CD142 (all monoclonal antibodies were purchased from BD Pharmingen™, Franklin Lakes, NJ, USA). Fluochromes were phycoerythrin (PE)-, fluorescein isothiocyanate (FITC)-, and phycoerythrin-Cy5 (PE-Cy5), and were appropriately combined to allow recognition of PMNL-PLT and MONO-PLT aggregates.

Erythrocytes were lysed by addition of FACS Lysing Solution (BD Pharmingen™) for 10 minutes and tubes were then centrifuged at 500 rpm for 10 minutes. Cells were resuspended in Dulbecco’s Phosphate Buffer Saline w/o calcium and magnesium (D-PBS; EuroClone S.p.A. Celfio, Milan, Italy) for the analysis. Cells were assessed by multiparameter flow cytometry that always included 2 physical parameters, that is, forward and side scatter (FSC and SSC, respectively) to establish the PMNL and the MONO region, and 3 fluorocenes. Samples were analyzed using a FACScan cytometer (BD Biosciences, San Jose, CA, USA), as detailed elsewhere.

Results were expressed as percentage of PMNL and MONO positive for CD41a, that is PMNL/MONO-PLT, respectively, and as percentage of PMNL and MONO positive for CD62P for aggregates with activated PLT, that is PMNL/MONO-PLT act. TF expression was measured as percentage of TF positive MONO.

RYGB

RYGB involves the use of a surgical stapler to create a small and vertically oriented gastric pouch with a volume usually <30 mL. The upper pouch is completely divided from the gastric remnant and is Anastomosed to the jejunum, 75 cm distally to the Treitz’s ligament, through a narrow gastrojejunal anastomosis in a Roux-é-Y fashion. Bowel continuity is restored by an entero–entero anastomosis, between the excluded biliary limb and the alimentary limb, performed at 100 cm from the gastrojejunostomy.

Body Composition

Body weight was measured to the nearest 0.1 kg with a beam scale and height to the nearest 0.5 cm using a stadiometer (Holatin, Crosswell, Wales, UK). Waist and hip circumference was measured using a tape measure around the waist and around the hips/buttocks with thin clothing on. The tape was pulled tight enough to compensate for the added girth of clothing. Hip circumference is defined as the distance around the largest extension of the buttocks.

Statistical Analysis

We hypothesized a longitudinal study in which there was a 30% reduction of leukocyte-PLT aggregates and TF expression

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at 6 months after surgery. Under this condition, 30 patients were needed to obtain a study with a statistical power of 80% for a 2-sided test with a significance level of 5% (EpiInfo 3.5.1, Freeware CDC).

Results are expressed as mean ± SD (95% confidence interval). We applied the nonparametric Wilcoxon matched pair test to assess the significance of intergroup differences. Mann–Whitney U test was used to compare controls and bariatric patients. The correlation analysis was performed using the Spearman nonparametric test. Multivariate models adjusted for oversampling was applied. We applied Friedman nonparametric test for repeated measures when evaluating longitudinal data in surgically treated obese patients, as detailed in the result section. Differences were considered significant at P value <0.05.

Statistical analysis was performed using IBM SPSS statistics (version 17.0) software.

RESULTS

Clinical characteristics of 58 obese patients at the enrollment and 30 healthy controls are shown in Table 1. Smoking habit was present in 14 obese patients (relative frequency, [r.f.] = 0.241) and hypertension in 22 obese patients (r.f. = 0.379). Hyperlipidemia was observed in 20 obese patients (r.f. = 0.345). The control group consisted of 30 healthy patients with a BMI < 25 kg/m².

PMNL-PLT and MONO-PLT aggregate frequency was increased in obese patients as compared with controls. The percentage of PMNL-PLT was 7.47 ± 2.45 (6.82–8.11)% vs 5.85 ± 1.89 (5.14–6.55)% in obese and nonobese patients, respectively (P = 0.001) and the percentage of MONO-PLT aggregates was 12.31 ± 7.33 (10.38–14.24)% and 8.14 ± 2.22 (7.31–8.97)% in obese versus nonobese patients, respectively (P < 0.001). The frequency of TF expressing MONO was 4.01 ± 2.22 (3.45–4.56)% in obese patients and 2.64 ± 1.65 (2.02–3.25)% in nonobese patients (P = 0.002).

The frequency of PMNL-PLT and MONO-PLT aggregates was positively correlated with TF expressing MONO (R² = 0.260, P = 0.049 and R² = 0.318, P = 0.015, respectively).

In obese smokers, PMNL-PLT aggregate frequency tended to be higher than in nonsmokers (7.69 ± 1.21 [7.24–8.14]% vs 4.46 ± 0.98 [4.08–4.84]%). No relationship between diabetes and PMNL-PLT and MONO-PLT aggregates was observed (not shown). Control smokers also showed a tendency toward a higher PMNL-PLT aggregate frequency than nonsmokers (9.62 ± 2.01 [8.51–10.73]% vs 5.56 ± 1.12 [4.96–6.16]%).

Thirty-one obese patients out of 58 underwent BS (14/17 M/F; mean age 42.8 ± 10.9 [39.95–45.7] years). Multivariate models adjusted for oversampling showed a correlation between BMI and PMNL-PLT aggregate frequency (adjusted R² = 0.105, P = 0.010).

The effects of BS on the baseline clinical and laboratory parameters were recorded at 3 (T₁), 6 (T₂), and 12 (T₃) months after BS and are shown in Table 2. There was a time-dependent reduction of BMI, waist and hip circumferences, CRP, glucose, HbA1c, total cholesterol, LDL cholesterol, and triglycerides during the follow-up. On the contrary, HDL cholesterol plasma level increased during the follow-up.

The effects of BS on PMNL-PLT and MONO-PLT aggregate frequencies and TF expressing MONO before and after BS are shown in Table 3. There was a time-dependent reduction of PMNL-PLT aggregate frequency that became statistically significant at T₃ after BS (7.58 ± 2.27 [6.75–8.42]% vs 4.49 ± 1.11 [3.93–5.01]%: P < 0.001). Similarly, MONO-PLT aggregate frequency showed a marked time-dependent

| TABLE 1. Main Characteristics of Obese and Nonobese (Controls) Patients |
|-------------|------------------|-----------------|------------------|
|             | Obese Patients   | Controls        |
| N           | 58               | 30              |
| Men/women   | 25 (0.431)       | 16 (0.533)      |
| Age, y      | 42.8 ± 10.9      | 39.4 ± 11.5     |
| Body mass index, kg/m² | 46.2 ± 6.9 | 23.39 ± 2.02 |
| Waist circumference, cm | 131.1 ± 13.8 | 83.4 ± 7.0     |
| Hip circumference, cm | 137.0 ± 14.6 | 85.1 ± 7.9     |
| Diabetes    | 26 (0.45)        | None            |
| Glucose, mg/dL | 109.5 ± 41.6 | In normal range |
| Glycated hemoglobin, % | 7.23 ± 3.86 | In normal range |
| Total cholesterol, mg/dL | 192.3 ± 35.4 | In normal range |
| HDL cholesterol (mg/dL) | 43.60 ± 8.72 | In normal range |
| LDL cholesterol, mg/dL | 120.49 ± 32.68 | In normal range |
| Triglycerides, mg/dL | 158.09 ± 71.40 | In normal range |
| C-reactive protein, mg/dL | 10.07 ± 6.12 | In normal range |
| Drug therapy |                  |                 |
| Antihypertensive | 22 (0.379) | None            |
| Proton-pump inhibitors | 9 (0.155) | None            |
| Statin       | 20 (0.345)       | None            |
| Antibiotics  | 3 (0.051)        | None            |
| Antidiabetic | 26 (0.448)       | None            |
| Analgesic    | 6 (0.103)        | None            |
| Other (dietary supplements) | 9 (0.155) | None            |

HDL = high-density lipoprotein, LDL = low-density lipoprotein. Quantitative variables are expressed as mean ± SD (95% confidence interval). Qualitative variables are expressed as absolute frequency (relative frequency).
TABLE 2. Changes in Metabolic Parameters of 31 Obese Patients at Baseline (T0) and at 3 (T1), 6 (T2), and 12 (T3) Months After Bariatric Surgery (Mean ± SD [95% CI])

|                      | T0               | T1               | T2               | T3               |
|----------------------|------------------|------------------|------------------|------------------|
| Body mass index, kg/m²| 45.8 ± 7.1 (43.2–48.4) | 38.4 ± 6.5 (35.7–41.0) | 37.1 ± 4.9 (34.4–39.8) | 34.2 ± 3.9 (30.9–37.4) |
| Waist circumference, cm| 131.2 ± 12.0 (126.8–135.6) | 116.9 ± 12.8 (111.73–122.07) | 116.1 ± 7.4 (112.2–120.0) | 104.5 ± 10.4 (99.0–110.0) |
| Hip circumference, cm | 137.2 ± 12.9 (126.8–135.6) | 128.2 ± 16.6 (121.5–134.9) | 119.1 ± 10.8 (113.3–124.9) | 113.8 ± 7.1 (110.0–117.6) |
| C-reactive protein, mg/dL | 9.06 ± 8.93 (4.29–13.82) | 6.03 ± 3.62 (4.10–7.95) | 0.42 ± 1.26 (-0.55–1.39) | 2.06 ± 2.99 (-0.46–4.57) |
| Glucose, mg/dL       | 108.69 ± 22.68 (96.60–22.68) | 76.12 ± 18.34 (66.35–85.90) | 73.67 ± 12.70 (63.31–83.43) | 63.88 ± 10.4 (50.82–76.93) |
| Glycated hemoglobin, % | 6.62 ± 1.01 (6.08–7.16) | 5.73 ± 0.56 (5.45–6.02) | 5.47 ± 0.49 (5.09–5.84) | 5.35 ± 0.57 (4.88–5.82) |
| Total cholesterol, mg/dL | 198.31 ± 32.97 (180.74–215.88) | 167.31 ± 46.22 (142.68–191.94) | 147.11 ± 31.75 (133.71–171.52) | 176.13 ± 47.01 (127.37–224.88) |
| HDL cholesterol, mg/dL | 44.31 ± 9.08 (39.47–49.15) | 47.00 ± 11.46 (40.89–53–11) | 46.22 ± 13.75 (35.66–56.79) | 51.38 ± 12.64 (40.81–61.94) |
| LDL cholesterol, mg/dL | 116.18 ± 31.56 (99.37–133.01) | 96.38 ± 36.46 (76.76–115.99) | 76.78 ± 19.71 (61.63–91.93) | 100.50 ± 40.80 (59.70–141.30) |
| Triglycerides, mg/dL  | 181.06 ± 71.29 (143.07–219.05) | 120.31 ± 55.53 (77.76–163.13) | 120.44 ± 31.72 (100.23–140.39) | 100.75 ± 30.17 (77.16–144.34) |

CI = confidence interval, HDL = high-density lipoprotein, LDL = low-density lipoprotein, SD = standard deviation. *P value was calculated versus baseline.

TABLE 3. Changes in PMNL-PLT and MONO-PLT Aggregate, TF Expression by MONO in Obese Patients at Baseline and 3, 6, and 12 Months After BS (Mean ± SD [95% CI])

|                      | T0               | T1               | T2               | T3               |
|----------------------|------------------|------------------|------------------|------------------|
| PMNL-PLT, %          | 7.58 ± 2.27 (6.75–8.42) | 6.61 ± 3.39 (5.21–8.01) | 5.81 ± 3.45 (3.16–8.46) | 4.47 ± 1.11* (3.93–5.01) |
| MONO-PLT, %          | 12.51 ± 8.22 (9.49–15.52) | 11.18 ± 5.15 (9.06–13.31) | 9.11 ± 3.00 (6.80–11.41) | 9.70 ± 1.70 (8.28–11.13) |
| PMNL-PLTact, %       | 1.94 ± 1.17 (1.51–2.37) | 1.61 ± 0.91 (1.23–1.99) | 1.50 ± 1.36 (0.45–2.55) | 1.63 ± 1.02 (0.78–2.49) |
| MONO-PLTact, %       | 5.22 ± 4.87 (3.43–7.00) | 5.06 ± 3.44 (3.64–6.48) | 3.31 ± 2.03 (1.74–4.87) | 4.65 ± 1.67 (3.21–6.05) |
| TF MONO, %           | 3.82 ± 2.04 (3.07–4.57) | 2.78 ± 1.66 (2.10–3.47) | 1.60 ± 1.69** (0.30–2.90) | 1.71 ± 0.54*** (1.45–1.97) |

BS = bariatric surgery, CI = confidence interval, MONO = monocyte, PLT = platelet, PMNL = polymorphonuclear leukocyte, SD = standard deviation, TF = tissue factor* P < 0.001; **P = 0.008; ***P = 0.001 (P value was calculated vs baseline).
reduction at \(T_2\) (12.51 ± 8.22 [9.49–15.52]% vs 9.11 ± 3.00 [6.80–11.41]%\) and \(T_3\) (12.51 ± 8.22 [9.49–15.52]% vs 9.70 ± 1.70 [8.28–11.13]%), that, however, failed to reach statistical significance. A similar tendency toward a progressive decrease of PMNL-PLTact and MONO-PLTact aggregate frequency was also observed.

TF expressing MONO frequency was significantly reduced at \(T_2\) (3.82 ± 2.04 [3.07–4.57]% vs 1.60 ± 1.69 [0.30–2.90]%; \(P = 0.008\)) and \(T_3\) (3.82 ± 2.04 [3.07–4.57]% vs 1.71 ± 0.54 [1.45–1.97%]; \(P = 0.001\)) after BS.

DISCUSSION

Obesity, and particularly an excess of central body fat distribution (central obesity), is an independent risk factor for arterial and venous thrombosis. Obesity may be linked to vascular disease through different mechanisms which include inflammation-related patterns.1–5 Consistently, body weight reduction following BS determines the normalization of inflammatory parameters and of coagulative activation.9,16–20

In this study, we investigated PMNL-PLT and MONO-PLT aggregate frequency and TF expressing MONO as markers of white blood cell activation, and CRP as inflammatory marker, in a group of obese patients, and we also evaluated how these parameters were modulated by the body weight reduction that follows BS.

We found that at baseline obese patients had a significantly increased level of PMNL-PLT and MONO-PLT aggregate frequency, as well as TF expressing MONO, as compared with healthy controls, thereby indicating that obesity brings about enhanced white blood cell activation. This view is in line with an early report demonstrating that in women with severe obesity PMNL-PLT and MONO-PLT aggregate frequency was increased in obese versus nonobese patients.24 How obesity favors PMNL and MONO activation is, however, unclear. The study of Das34 is one of the first to suggest that obesity is an inflammatory condition. It is likely, therefore, that a chronic inflammatory status which, in turn, induces PNML and MO activation can be the consequence of obesity. Supporting this view, the administration of a fatty meal, an experimental condition that mimics an acute inflammatory response, has been reported to be accompanied by an increased frequency of leukocyte-PLT aggregates in humans.29 In obese smokers, the PMNL-PLT aggregate frequency was increased as compared with obese nonsmokers. The same tendency was observed in healthy controls, implying that the smoking habit per se can determine PMNL-PLT aggregate formation. This view is in agreement with a previous study in an animal model showing that cigarette smoke induces PMNL-PLT and MONO-PLT aggregate formation.52 In addition, other studies demonstrated that cigarette smoking evokes an inflammatory response in both humans and animal models.53,54 Collectively, these data imply that the smoking habit contributes to the raise of frequency of PMNL-PLT aggregates through the induction of an additional chronic inflammatory status.

Unexpectedly, the frequency of PMNL-PLT and MONO-PLT activated aggregates was comparable between obese and control patients, and was uninfluenced by BS. This observation may merely reflect the inadequacy of the method we used here for assessing PLT activation. PLT activation was assessed by measuring the level of expression of CD62P by PLTds bound to PMNL and MONO. CD62P is a PLT adhesion protein which is involved in the binding of PLTs to the leukocyte coreceptor P-selectin glycoprotein ligand-1. Thus, we can infer that this interaction could have masked the antibody binding site of the CD62P molecule and hampered CD62P detection.

TF is the primary cellular initiator of the blood coagulation cascade.36,37 Previous studies have shown an association between enhanced TF expression on MONO and leptin, an adipocyte-derived mediator of long-term regulation of energy balance.15–16,30 We did not assess leptin in our series. However, because leptin levels in plasma are acutely increased during infection and inflammation,25,46 as well as in obesity,26 it is possible that the enhanced frequency of TF expressing MONO in our obese patients might reflect an increased level of plasma leptin. Thus, leptin-induced MONO activation might constitute an additional link between inflammation and haemostatic activation in obesity.

The clinical relevance of the indices of white blood cell activation we have studied has been recently reported.25–27,49 For example, an increased number of circulating PMNL-PLT and MONO-PLT aggregates has been observed in patients with different CVDs, such as stable and unstable angina, and myocardial infarction, and in patients undergoing percutaneous coronary interventions and heart valve replacement.26 In addition, PMNL-PLT and MONO-PLT aggregates are a predictive index of acute reocclusion following coronary angioplasty.42,43 Thus, although somewhat technically demanding, assessment of PMNL-PLT and MONO-PLT aggregates by flow cytometry may represent an additional useful parameter for risk assessment in a number of cardiovascular and inflammatory diseases.

BS determined an improvement of a series of parameters, including BMI, waist and hip circumference, total cholesterol, LDL and HDL fractions, glycemia and glycated haemoglobin, in accordance with previous literature.17–22 In our study, there was also an early decrease of CRP level that occurred quite rapidly and well before a substantial body weight reduction. The exact explanation of this rapid CRP decrease will require further investigation. However, because CRP is released by the liver following secretion of certain cytokines, among which there are IL-6 and TNF-α by macrophages and adipocytes, we can conclude that the rapid CRP normalization can be related to a rapid reduction of the plasma level of these cytokines.

Perhaps the most interesting finds of the present work was the progressive time-dependent reduction of PMNL-PLT and MONO-PLT aggregate frequency after BS. These data are in contrast with a previous study that showed that PMNL-PLT and MONO-PLT aggregate frequency did not vary significantly in patients undergoing BS 1 year after surgery.44 We can only conjecture as to why our results differ from that previous study.44 Comparisons are difficult because of factors such as the type of BS (gastric banding or gastric bypass in Ref. [44] and gastric bypass in our study) and inclusion of only women in that study,44 whereas our patient population consisted of both men and women.

Although PMNL-PLT and MONO-PLT aggregate formation is favored by the presence of the inflammatory status typical of obesity, it is possible to envisage that PMNL-PLT and MONO-PLT aggregates can contribute to the development of this inflammatory status. In this context, experimental studies have shown that leukocyte-PLT interaction triggers delayed responses in leukocytes by inducing the expression of certain transcription factors such as NF-kB, which, in turn, activates gene transcription for the synthesis of proinflammatory molecules, such as MCP-1, IL-8, and IL-1β. These soluble factors are critical for shifting the leukocyte phenotype toward an inflammatory and prothrombotic status.29,30 Consistently,
previous studies have shown that the plasma level of IL-8 and IL-1β are increased in obesity and associated with other obesity-related parameters.\textsuperscript{55,56} Thus, we can hypothesize that PMNL-PLT and MONO-PLT aggregates produce obesity-related cytokines which, in turn, contribute to leukocyte activation and, possibly, to PMNL-PLT and MONO-PLT aggregate formation and enhanced MONO activation. Interestingly, early studies have also shown that plasma levels and hepatic expression of IL-8 and IL-1β proinflammatory cytokines decrease in concert with BMI, metabolic parameters, CRP, and leptin after BS.\textsuperscript{18,20} thereby further supporting the hypothesis of a strong causative association between visceral adiposity, release of proinflammatory cytokines, and leukocyte-PLT aggregate formation.

A possible additional mechanism linking inflammatory response and coagulative activation is the increase in TF expressing MONO. It was shown that adipocyte-derived leptin\textsuperscript{50} facilitates TF expression by MONO, in accordance to what we found in our obese cohort. Moreover, a previous study has shown a strong link between decrease in leptin level and in BMI after BS.\textsuperscript{51}

In conclusion, weight loss induced by BS (RYGB) leads to the reduction of the systemic inflammatory status, as revealed by the normalization of CRP, PMNL-PLT, and MONO-PLT aggregates, and TF expression by MONO. Although future studies including the measurement of plasma level of inflammatory cytokines, like TNF-α and IL-6, are needed to provide a mechanistic explanation for these observations, our data confirm that obese patients show a proinflammatory and prothrombotic profile. The increase in leukocyte-PLT aggregates seems to indicate the existence of a novel link between inflammation and thrombosis.

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