AUGMENTATION OF OSTEOGENESIS WITH THE USE OF AUTOLOGOUS GROWTH FACTORS (AGF)
A PRELIMINARY CLINICAL AND LABORATORY REPORT

INTRODUCTION
Tissue injury leads to the migration and deposition of platelets and subsequently to release of growth factors at the wound site. Among the growth factors released from the platelets, the Platelet-Derived Growth Factor (PDGF) and the Transforming Growth Factor-beta (TGF-β) are considered to be particularly important in the bone repair process. PDGF is one of several osteogenic factors which affect bone growth and fracture healing. PDGF is a glycoprotein which exists mostly as a heterodimer of two chains A, B (PDGF-AB). Transforming Growth factor beta-2 (TGF-β2) is a more protean and generic factor involved with connective tissue repair and bone regeneration [1,2].

The purpose of our study is to evaluate the new bone formation in cases of long bone defects and spinal fusions using the use of AGF-bone graft construction (clinical study) and to estimate the concentrations of PDGF-AB and TGF-β2 during the procedure (clinical study).

PATIENTS AND METHODS
I. CLINICAL STUDY: After approval from the institutional review board and obtaining patients’ informed consent, the AGF-bone graft combination was used in 19 patients with long bone defects (11) and spinal fusion (8). Five patients with long bone defects and 2 with spinal fusion did not have an adequate follow-up period and were excluded from the present study. The remaining 14 patients had mean age of 44 years. Defects were located at the tibia (6) and femur (2) and resulted from gunshot injuries (1), high velocity fractures (1) and nonunions of 8-15 months duration (6 patients). Spinal fusion was necessary for the treatment of spinal stenosis (3) or fractures (T12, L1, L2) (3).

Two main steps of the procedure were the following:

1. Blood collection: One unit of whole blood (450 ml) was drawn in the specific CPD blood bag (suitable for buffy coat production), within 24 hours prior to the procedure.

2. Buffy coat preparation: The collected unit of whole blood was centrifuged (Heraeus 6000D) at 2700 rpm for 8 minutes and separated into 3 fractions: platelet-poor plasma (PPP), buffy coat (BC) consisting of platelets and white blood cells, and red blood cell pack (RBC). The BC bag, stored at 4°C, should be used within 24 hours. BC contained a volume of 180 mL with hematocrit not greater than 5%, and contained at least 400,000/mL platelets and 10,000/mL white blood cells.

3. AGF processing: The BC was further processed into an ultra-concentrated platelet solution (Interconcentrator, Interpore Cross™) and 50-60 ml of AGF concentrate were obtained.

4. Long bone defect-Spinal fusion preparation: In the long bones, debridement to viable bone was followed by lavage and dead space management when necessary. The stability of fixation was confirmed in cases of pre-existing external fixation and was secured in cases of absent or inadequate fixation. Patients with spinal stenosis underwent laminectomy and posterior stabilization and patients with spinal fractures underwent posterior instrumentation.

5. Autologous bone graft harvesting: Iliac crest or iliac bone graft concentrate was obtained from all the patients of our series. Clinical platelet counts of patients were obtained with a Coulter Act-10 counter from blood (patient baseline). Aliquots were taken at each stage (whole blood, buffy coat, AGF, wound drain) and analyzed for TGF-β2, PDGF-AB concentration and platelet counts. Enzyme-Linked Immunosorbant Assays (ELISA) were performed to quantify concentrations of active PDGF-AB and TGF-β2 (Quantikine Immunoassays, R and D system). Optical densities of specimens were read on a microtitre plate reader at 450nm with correction at 540nm.

RESULTS
I. CLINICAL: Mean follow up time was 9 months (6-12). Signs of bone union were apparent in radiographs 3-6 months after the index procedure. External fixators were removed from the tibiae 5-8 months postoperatively. Clinical examination confirmed the absence of pain in the area of bone defect/spinal fusion.

II. LABORATORY: In our series, the average platelet count increased from 212x10^8 cells/ml to 680x10^8 cells/ml in the buffy coat and to 1280x10^6 cells/ml in the AGF concentrate, resulting in a 604% increase. Preliminary studies showed a 480% increase of PDGF-AB levels in AGF concentrate comparing to whole blood (baseline). Furthermore, a 320% increase of TGF-β2 levels in AGF concentrate comparing to whole blood levels was determined. TGF-β2 and PDGF-AB levels were also detected in samples collected from the wound drains, in increased levels comparing to the AGF concentrates (Figure 1). Additionally, wound drain samples from 3 patients who underwent surgical procedures (tibial osteotomies) without AGF gel application were also evaluated and TGF-β2 and PDGF-AB levels were lower compared to the wound drain samples from the patients of our series.

DISCUSSION
In all cases the clinical results were very encouraging with augmented osteogenesis, whereas the laboratory results (increased values of TGF-β2 and PDGF-AB in subsequent stages of the procedure) practically predicted the clinical success.

Marx et al [3] and Lowery et al [4] have reported an average 338% and 575% platelet count increase in AGF concentrate respectively, comparing to baseline values. Additionally, a 546% and 380% increase of TGF-β2 and PDGF levels respectively, comparing to baseline values, has been also documented [4]. The values determined in all the patients of our series were very close to the ones reported above [3, 4]. In our series, TGF-β2 and PDGF-AB average values in wound drain samples were higher comparing to the control wound drain samples (procedures without AGF) and to the AGF concentrate. Although there are no relevant literature reports to support this observation, we believe that the increased values in the wound drain samples of AGF-treated patients, reflect a higher cleavage of growth factors both from the applied AGF-bone graft gel and from the platelets migrating to the wound site.

Although our results are preliminary we consider the use of autologous growth factors a highly effective technique for the augmentation of osteogenesis.

REFERENCES

FIGURE 1