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The evolution of callus formation after stimulation with bone graft and bone marrow aspirate from iliac crest

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SUMMARY

Background. The experimental research studies the behaviour of open fracture site after stimulation with cortical-cancelleous graft and medular aspirate from iliac crest.
Methods. The animal used was home rabbit, because he has an anatomy which permit the bone study. The animals were divided in 4 study groups due to the callus stimulation method and the aspect of fracture site (simple, comminuted). For each group of study, the evaluation was made separately, due to the osteosynthesis type, resulting subgroups of reasearch.
Results. The evaluation of the results was made by clinical, histological and radiological study comparing the research groups, at established periods of time. The final evaluation was made at 6 weeks, by calculating clinical, radiological and histological scores with high specificity, which permited a correct interpretation of the obtained results.
Conclusions. The study confirms the role of the bone marrow and of the cortico-cancellous bone graft in ostheogenesis, in which the osteoprogenitors cells survive permamently and have an important role to stimulate and to accelerate consolidation.
Keywords: callus stimulation, open fracture, bone marrow, bone graft

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BACKGROUND

The aim of the experimental research was to follow the evolution of open fracture site after stimulation with cancellous bone graft and bone marrow arpirate from iliac crest and to evaluate the capacity to stimulate and accelerate osteogenesis.

MATERIAL AND METHODS

The lab animal we used in our research was the domestic rabbit because of his anatomy which permited us to make a bone study. The clinical experiments were made on 19 young rabbits (30 cases - fractures), with age between 6 and 8 months, weight between 1500 and 2000g, with the support of animal laborator ("Biobasis") of Faculty of Biology from "Ovidius" University of Constanta, Romania.

The research on the rabbits was made according to European Community Standards and to the World Health Organization guidelines on animal experimentations.

The bone graft was taken from the iliac crest of the same rabbit, in the same surgery time with osteosynthesis.[1,2]

For the conception of this study and based on the informations found in the literature, we started from the ipothesis that the open fracture site will heal faster after stimulation with bone graft and bone marrow and we evaluated:

1. the influence of stimulation method of consolidation upon the evolution of fracture
2. the influence of osteosynthesis type upon consolidation
3. the evolution of consolidation depending of fracture type (simple/comminuted).
4. radiological evaluation of fracture site evolution at 2, 4, 6 weeks

5. histological evaluation of fracture site evolution at 7, 14, 30, 42 days
6. the evaluation of the results at 6 weeks: clinical, radiological and histological results [3, 4, 5, 6]

Scoring criteria:

A. Clinical
   I. Full weight bearing/walk on operated limb (time interval)
      – Absent – 1
      – Present – first day 4
      – first week – 3
      – at 3 weeks - 2
   II. Pain – present -1 / absent -2

B. Radiological – new bone formation (time interval)
   – bone resorption – 1 (poor)
   – moderate – 2
   – callus cortical bridging, fracture line well visible – 3
   – callus cortical bridging, fracture line poor visible – 4 (excellent)

C. Histological – bone defect – 1
   – fibrosis – 2
   – fibro-cartilage – 3
   – bone – incomplete bridging – 4
   – bone – complete bridging – 5
   – medullary duct formation (bone marrow) – 6.

The scoring system we used served as objective indicator to appreciate the groups we studied, to evaluate the surgical techniques and the method of promoting the consolidation applied to our group of animals. [7,8]

RESULTS

The surgical approach to the femur represented an agression for the soft tissue which affected the local vascularization, but not like an

Tab. 1. Group I (control group) without stimulation of consolidation

Group	Subgroup	Osteosynthesis type	No cases
I – 15 cases	IA	K-wire	11
	IB	External fixator	4

Tab. 2. Group II – fractures upon was applied the method of callus stimulation

Group	Subgroup	Osteosynthesis type	No cases
II – 15 cases	IIA	K-wire	11
	IIB	External fixator	4

Tab. 3. Group III – simple fractures

Group	Subgroup	Callus stimulation	Subgroup depending on osteosynthesis type	No cases
III – 18 cases	IIIA	– – –	III Aa. K-wire	10
			III Ab. External fixator	1
	IIIB	+++	III Ba. K-wire	6
			III Bb. External fixator	1

Tab. 4. Group IV – comminuted fractures

Group	Subgroup	Callus stimulation	Subgroup depending on osteosynthesis type	No cases
IV – 12 cases	IVA	– – –	IV Aa. K-wire	1
			IV Ab. External fixator	3
	IVB	+++	IV Ba. K-wire	5
			IV Bb. External fixator	3

Group I – Subgroup IA

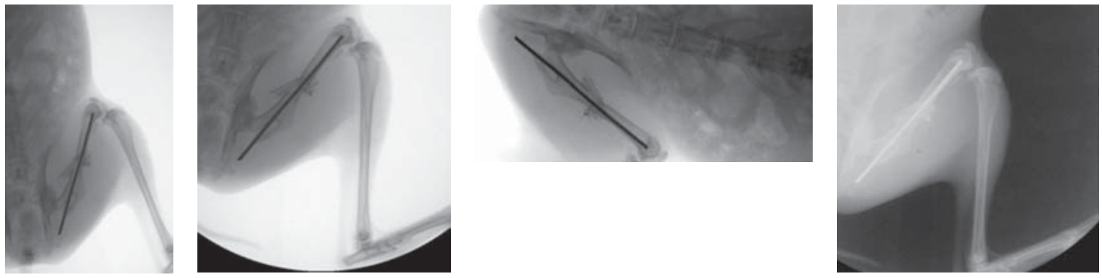

Fig. 1. Case 1: aspects postoperatively, at 2 weeks, at 4 weeks and at 6 weeks. Radiological score 3

Subgroup IB – osteosynthesis with external fixator

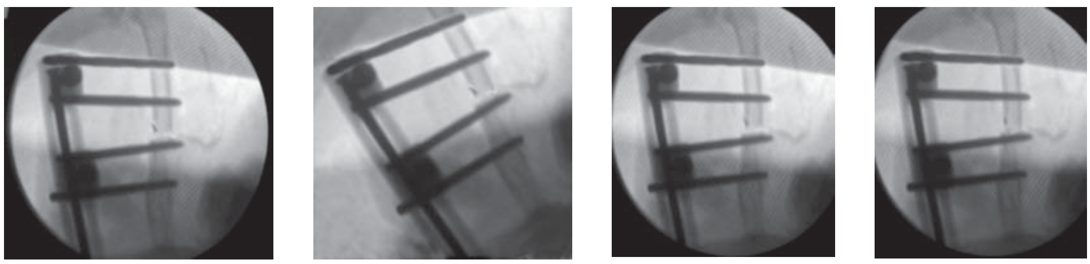

Fig. 2. Case 2: aspects postoperatively, at 2 weeks, at 4 weeks and at 6 weeks. Radiological score 2

Group II – Subgroup IIA

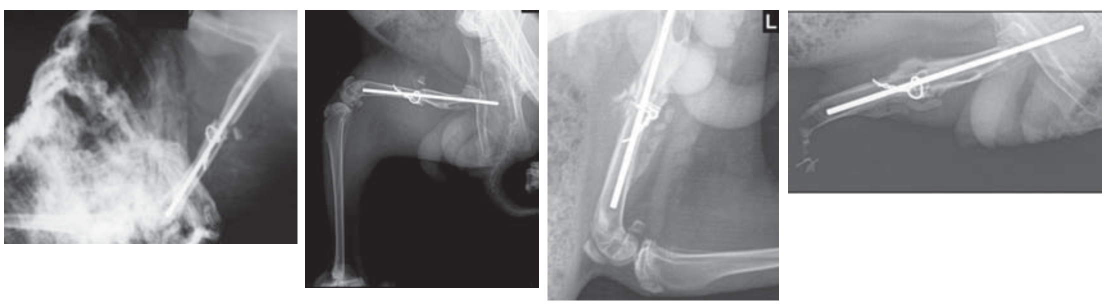

Fig. 3. Case 3: aspects postoperatively, at 2 weeks, at 4 weeks and at 6 weeks. Radiological score 4

Subgroup IIB – osteosynthesis with external fixator

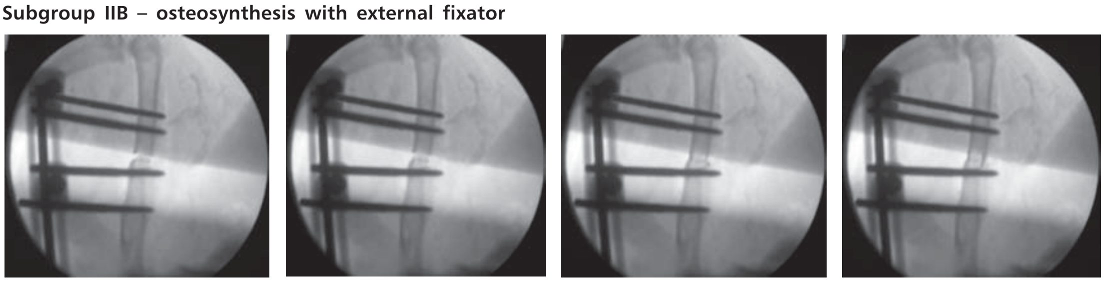

Fig. 4. Case 4: aspects postoperatively, at 2 weeks, at 4 weeks and at 6 weeks. Radiological score 3

open fracture type III for example, when the wound is contaminated and affects directly the consolidation.

Our casuistry was divided in 4 groups depending on the method of callus stimulation and the fracture site aspect (simple or comminuted). In each group, the evaluation was made distinctively, depending on the osteosinthesys type, resulting separate subgroups of research. The group II is compared with group I (control group). (table 1, 2)

Research groups III and IV were divided in 2 subgroups, depending on the application or not of the stimulation method of consolidation, each group being also divided in other 2 subgroups, depending on the osteosynthesys type we applied. The group IV is compared with group III. (table 3, 4)

In case of the open fracture at human patients, the preferred ostheosynthesys methods are centromedullary ostheosynthesys and external fixation, but the preference for one or another are still debated. Osteosynthesys with K-wire was made in normal condition on the animal patient, being a method used as routine in veterinary traumatology. External fixation with minifixator was difficult due to the special anatomic conditions, as the femur of rabbit has a thin, hard, but very fragile cortical. (figure 1, 2, 3, 4)

DISCUSSIONS

The bone marrow was applied in 2 ways: bone marrow aspirated from iliac crest and implemented at the fracture site and bone marrow with cancellous graft together implanted at the fracture site. The graft was applied directly at fracture site after the osteotomy was done (fracture), but bone marrow was introduced under

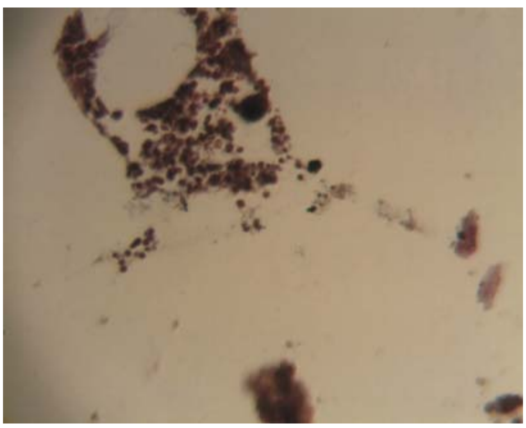

Fig. 5. Hematogenous marrow with cellular component of all series and erythroblastic hiperplasy (HE, 40X)

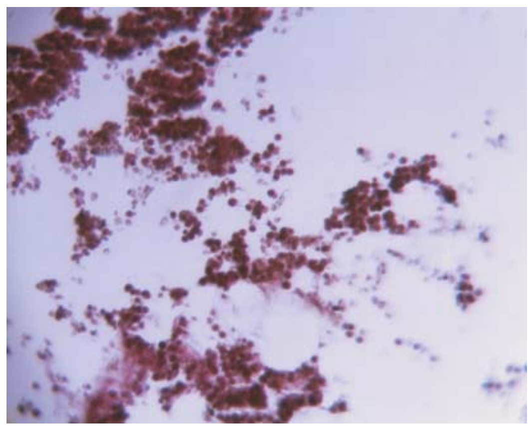

Fig. 6. Cancellous bone with high number of erythroblast cells (HE, 40X)

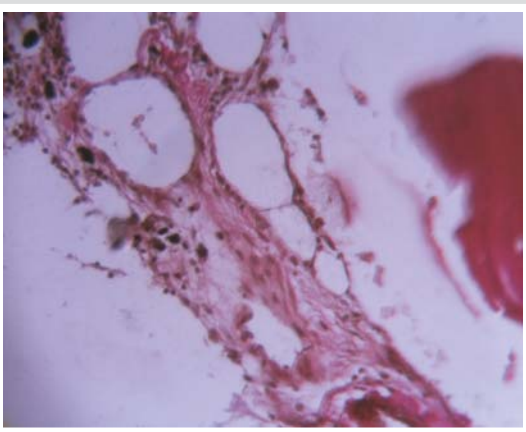

Fig. 7. Cancellous bone with bone marrow, rare erythoblastic cells, granulocytis, trabecullar bone, adypocites. (HE, 40X)

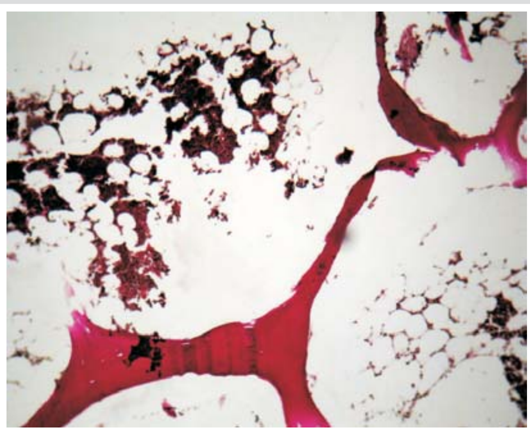

Fig. 8. Cancellous bone with bone marrow. (Van Gieson, 10X)

radiological control after the suture of the wound. (figure 5, 6, 7, 8)

The radiologc exam consisted of simple incidence X-ray postoperatively, at 2, 4 and 6 weeks. The radiologic score was calculated by the evaluation of the fracture at 6 weeks after surgery.

The medium radiologic score of group I (control group) was 2.4, while the radiologic scor for group II, in which the consolidation was stimulated was 3.6, significat greater than control group. It is interesting to find out if the efficacity of the method is the same in case of selective evaluation of casuistry, depending on the osteosynthesis method. The median radiologic score of subgroup IA, fractures of which we applied centromedullary osteosynthesis with K-wire in the control group, was 2.6, and the median radiologic score of group IIA, fractures of which we applied centromedullary osteosynthesis with K-wire in the group we applied the callus stimulation, was 3.7, significant superior. In case of subgroup IB where we used external fixation in the control group, the median score was 2.5, and in the subgroup IIB with external fixation and the stimulation of callus, the score was 3.3, a higher score too. (table 5, 6)

We can conclude that stimulation of callus through the method we used influenced positively the evolution of fractures by formation of the new bone in a shorter period of time and with better quality as control group, no matter the osteosynthesis type we choose.

It is interesting the comparative evaluation of the median radiologic score of group III, simple fractures, with group IV, comminuted fractures. The radiologic score of group III is 3, lower than the median radiologic score of group IV which is 3.3. At first view is a result which make difficult to evaluate correctly because we expected that simple fractures to have a better evolution of callus formation than comminuted fractures. Annalysing the two groups, these results can be explained by the fact that in 66.7% of the comminuted fractures was applied the callus stimulation method, higher percent than 38.9% of simple fractures of which the method was applied.

The clinical score was calculated by tracing the parameters represented by pain, weight-bearing and walk on the operated limb. Pain codificated by her presence of absence, evaluated by the vivacity of the animal at 24-48 hours, weight-bearing on operated limb, defense when the limb was touched on the operated site.

The group I (control group) has a median score of postoperatively mobility of 1.6, and a median score of pain of 1.26. The group II has a median score of mobility of 2.93 and a median score of pain of 1.6. The group II, with callus stimulation, has a superios score of

Tab. 5. Median radiologic scores – groups

Group	I	II	III	IV
Score	2.6	3.6	3	3.3

Tab. 6. Median radiologic scores - subgroups

Group	IA	IB	IIA	IIB
Score	2.6	3.7	2.5	3.3

Tab. 7. Clinical score – median comparative aspects

Group	I	II	III	IV
Mobility	1.6	2.93	2.22	2.41
Pain	1.26	1.6	1.5	1.5

Tab. 8. Histological score – comparative aspects

Group	I	II	III	IV
Score	3.8	6.5	4.6	4.2

mobility than control group, with direct relation to the radiological score which was better for group II comparative to group I, due to a quicker formation of callus, so a better stability of the fracture site than control group, which permitted an early mobilisation.

Through the comparative study of group III and IV we can appreciate the influence of the fracture type, simple or comminuted, upon the two indicators of clinical score. Regarding the median score of the pain, there is no difference between the two groups, an identical score of 1.5. The median score of mobility as the radiological score is better in the comminuted fractures group because, as we showed previously, the number of fractures we stimulated consolidation was bigger in the comminted group (66.7%) as the simple fractures group (38.9%). (tabel 7)

The samples of tissue were fixed in formaldehyde 10%. The bone tissue was decalfied in EDTA 4-6 hours. After dehidratation with etylic alcohol and clarified with toluene and included in paraphine. The histological sections were cutted with microtome 2μm thin, than colored and studied by microscope. The coloration we used were hematoxilin-eosine and van Giesom.

The histological study of the samples taken from the fracture site consist of peiosteum, cortical bone, cancellous bone, bone marrow for each group and subgroup. The qualitative evaluation of the histological sections from the fracture site followed two directions of research: evolution of the fracture site at 7, 14, 30, 42 days and the appreciation of the histological score at 42 days. The histological score permited an objective evaluation of callus evolution at 6 weeks from the fracture.

For the group I we appreciated the histological score in 40% of cases, for the group II in 33.3% of cases, for group III in 46.6% and for group IV in 26.6% of cases.

The group I had a median histological score of 3.8, while group II had a median histologic score of 6.5, almost double, so obviously a faster consolidation of the group II than control group.

Regarding the evolution of fracture site depending of the presence of comminution or not, we observe a better evolution in the group of simple fractures, group III, which had a median histologic score of 4.6 and the group IV which had a lower histologic score 4.2. These differencies were not significant statistically because the results were influenced by the presence unequally as number of cases of the callus stimulation method. In the group III, from 7 cases aleatory chosen, in 2 cases was aplied the stimulation method (28.6%), while in group IV, from 4 cases aleatory chosen, in 3 cases we aplied the callus stimulation (75%). So, in the comminuted fractures group IV, the histologic score was higher because of callus stimulation method. (table 8)

The results obtained in group II prove an increase activity of osteogenesis. That thing is higlighted by structures which indicated an active process of osteoformation: new lamellar bone formation, multiple osteoblasts situated liniary at the mineralisation frontline, young endhotelian cells, new vessels with protuberant endhotelian cells inside the lumen. The osteoblasts situated at the mineralisation frontline have big nucleus, hypercromatic which indicate a cellular hyperactivity. (figure 9, 10)

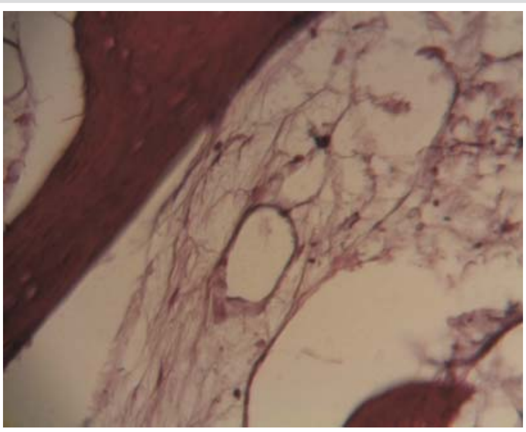

Fig. 9. Cancellous bone graft at 30 days. Lamellar bone tissue with rare osteocytes. Low hematogenous celularity. Cartilaginous osification. (HE, 20X)

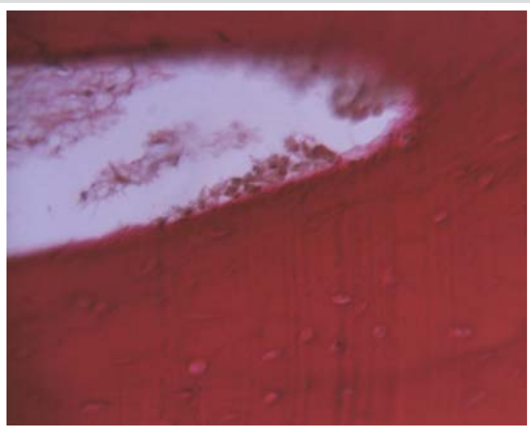

Fig. 10. Integrated bone graft at 42 days. Lamellar bone tissue with gaps and osteocytes. Osteoblasts at mineralization frontline. (HE, 20X)

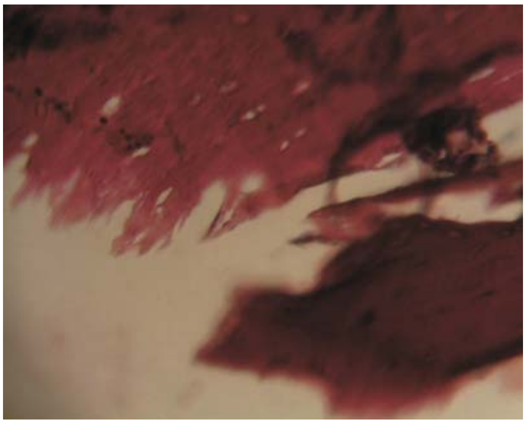

Fig. 11. Bone graft complete integrated at 42 days. New bone formation. Well structured bone lamellas. Reshuffling process. (HE, 20X)

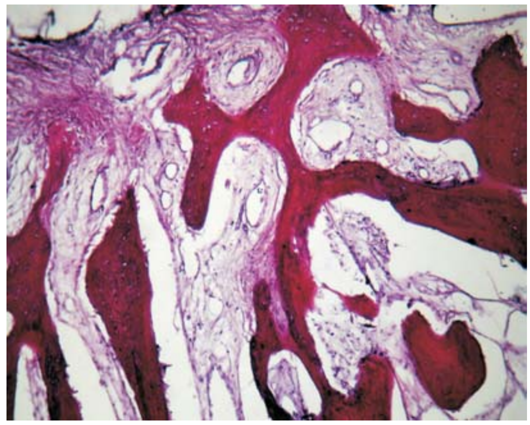

Fig. 12. Consolidated fracture site, integrated bone graft, new bone. (HE, 10X)

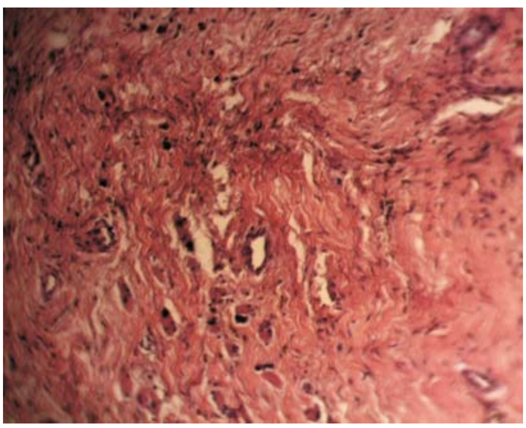

Fig. 13. Aspects at 42 days. Callus with enchondrale ossification, perichondral granulation tissue, interstitial haemosiderine deposit. (group I) (HE, 40X)

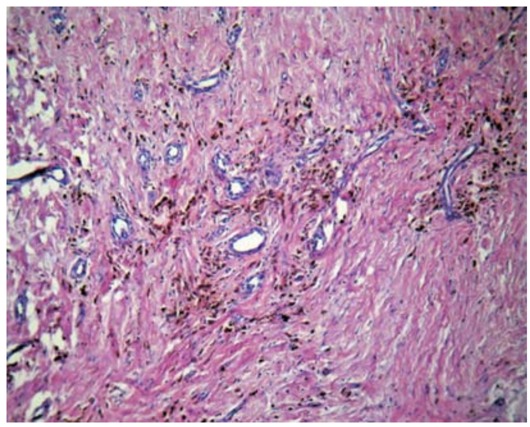

Fig. 14. Aspects at 42 days. Callus with enchondrale ossification, new capillars, interstitial haemosiderine deposit. (group I) (HE, 10X)

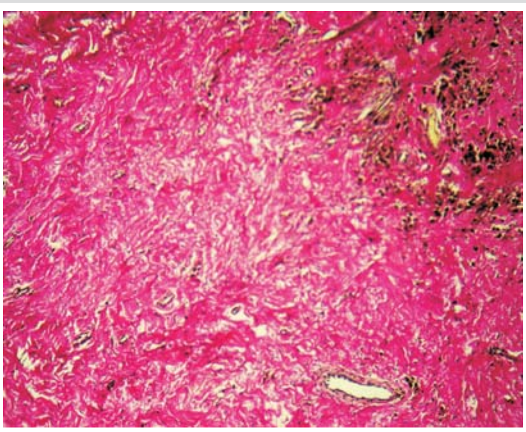

Fig. 15. Aspects at 42 days. Callus with enchondrale ossification, new capillars, interstitial haemosiderine deposit. (group I) (Van Gieson, 10X)

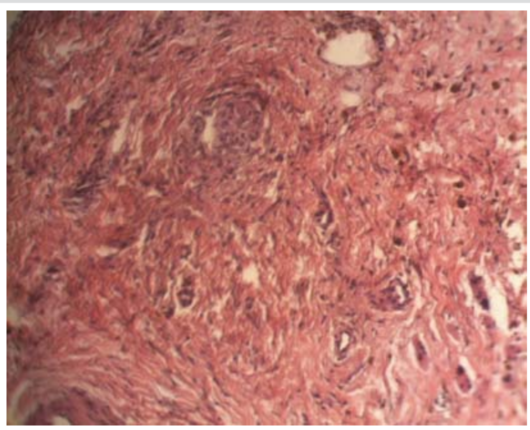

Fig. 16. Aspects at 42 days. Enchondrale ossification, cartilaginous tissue, colagen fibers and new capillars formation. (group I) (HE, 40X)

The periosteum taken from the fracture site, stimulated by the bone graft had a slow evolution to fibrinous degeneration seen at 30 days or slow evolution to ossification, with new bone formation.

The bone marrow had a histological evolution very good at the level of bone graft aplied at the fracture site, without degeneration in the cases in which the osteosynthesis was stable or small areas of fat or hyaline degeneration, in the cases in which osteosynthesis was not stable or the graft migrated from the fracture site. (figure 11, 12)

In the group II, the bone marrow had an increased cellularity near the fibrinous clot, element which indicates the evolution to osteoblastic cell, without degeneration, especially in the cases in which the osteosynthesis was stable. The new formed cartilaginous tissue presented hyperemic vascularity and vessels with thick walls. In the cases osteosynthesis wasn't stable we observed on the histologic sections from the fracture site, necrotic areas followed by fat metaplasia and calcium deposits, with the disapearance of the vessels.(figure 13, 14, 15, 16)

The histological healing of the fracture site was made with a voluminous callus, with a cartilaginous component in case of K-wire osteosynthesis, with definitve callus, even reshuffled in some cases, at 42 days, for group II of fractures in which callus stimulation method was applied. In case of external fixation, consolidation was made through enchondrale osification,

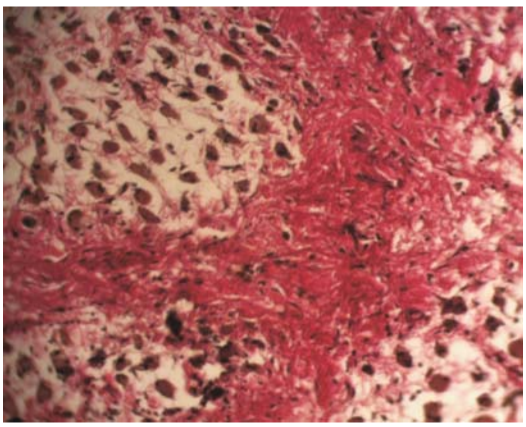

Fig. 17. Aspects at 42 days. Callus with enchondrale ossification, perichondral granulation tissue, lamellar bone tissue with osteocytes in gaps. (group II) (HE, 20X)

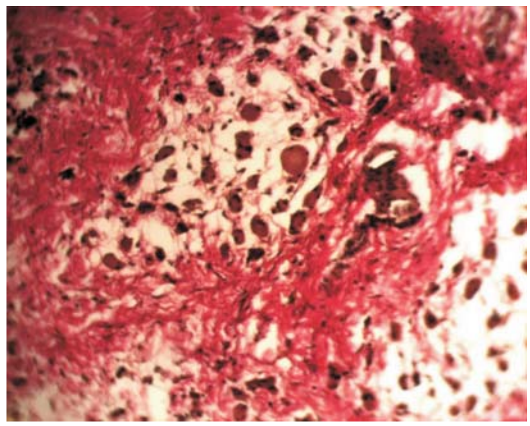

Fig. 18. Aspects at 42 days. Callus with enchondrale ossification. Vascular hyperplasy, thrombes, granulation tissue. Trabecular young bone. (group II) (HE, 20X)

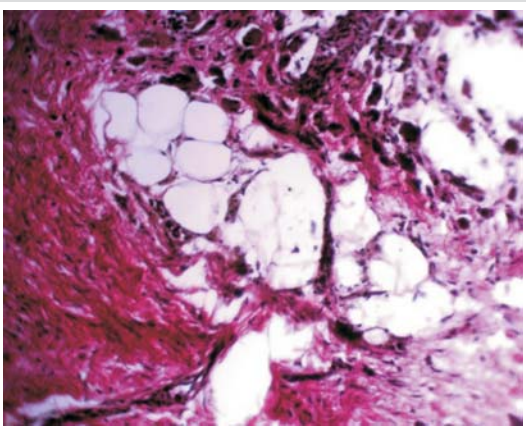

Fig. 19. Aspects at 42 days. Callus with enchondrale ossification. Fibro-conjunctive tissue and difuse colagen, lammelar bone tissue with osteocytes, new capillars, adypocites. (group II) (HE, 40X)

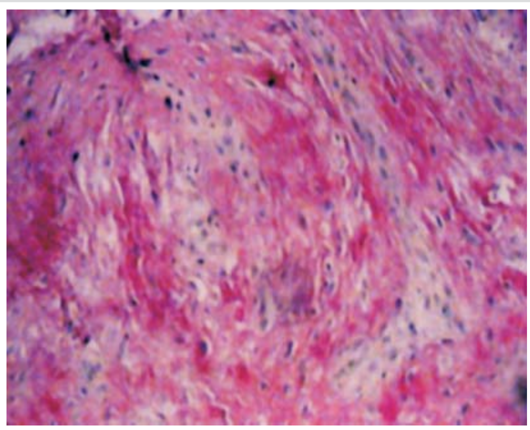

Fig. 20. Aspects at 42 days. Neoformation vessels in longitudinale section, endhotelial cells with proeminent nucleus. (Van Gieson, 10X)

but with a less voluminous callus. In the case of group I, enchondrale osification was longer as 42 days, if we take as final reference point the reshuffling period. (figure 17, 18, 19, 20)

The new formed bone tissue was observed at 7 days which produced the loosening of normal arhitecture of marrow and increased cellularity. The histologic evolution with the formation of new bone immediately after trauma, more obvious at group II, confirmed the existence of the cells with osteogenic potential at the level of bone graft. The formed bone tissue and osteoid tissue we observed at 7 and 14 days, together with cartilaginous tissue and well contoured cappilares at 30 days. The primitive callus had at first the characteristics of unitive and fixation callus, with discrepances generated by the osteosynthesis types: more voluminous and anarchical in case of osteosynthesis with K-wire and with low volume in case of external fixation. The medular channel was permanently well contoured, with presence of vascularity in both types of osteosynthesis. The increased answer of cellular response at the period of times established for the histological evaluation, was in direct relation with the presence of bone graft and bone marrow aspirate, being more actively at group II. (figure 21, 22)

The cancellous bone graft had a good histologic evolution, with the maintain of the macroscopic structure, with surviving of osteoblasts at the edge the graft at 7 days, with small areas of degeneration at 14 days, but with integration of the graft at 30 days.

At the examination of the histological sections from the fracture site, especially for group II, at 30 and 42 days from the surgery, there were noted the following results:

1. young bone with hyperplasia of osteocytes and osteoblasts which form a continuous line at the border with new bone.
2. the osteoblasts which constitute a new line at the level of mineralisation frontline have big and hypercromatic nucleas, with indirect sign of inactivity.
3. new lamellar bone with osteocytes disposed in groups, osteocytes hyperplasia.
4. new lamellar bone with fibroconjunctive tissue and neoformation vessels, in different sections, endhotelial cells with proeminent nucleas into the lumen.

CONCLUSIONS

Combined use of bone marrow aspirate with cancellos bone graft, harvested from the iliac crest, as method of callus stimulation in open fractures, represents an usefull method in the treatment of such cases. The consolidation period of time of the fractures in which the method was applied was shorter than in control group.

The study confirms the role of bone marrow and cancellous bone graft in the reparatory osteogenesis, in which the osteoprogenitory celularity survives constantly and has a decisive role in the stimulation and acceleration of consolidation.

The obvious higher histologic score of the group we aplied the osteogenesis stimulation than the control group is an important argument of the value of bone marrow and bone graft contribution in case of open fractures, no matter the aspect of fracture site (simple or comminuted), in case of a stable osteosynthesis. The histologic evolution with the formation of new bone immediately posttraumatic, more obvious in group II, confirme the existence of the cells with osteogenic potential at the site of the graft.

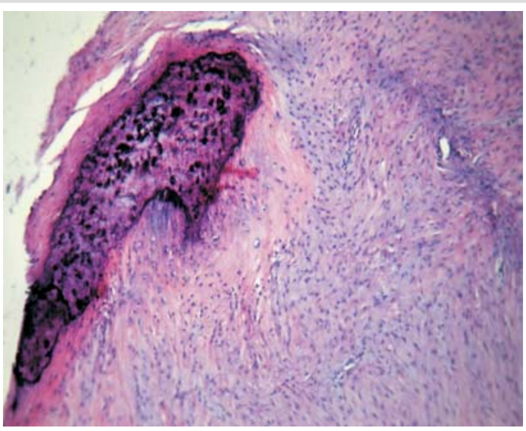

Fig. 21. Aspects at 42 days. Callus with enchondrale ossification. (group II) (HE, 10X)

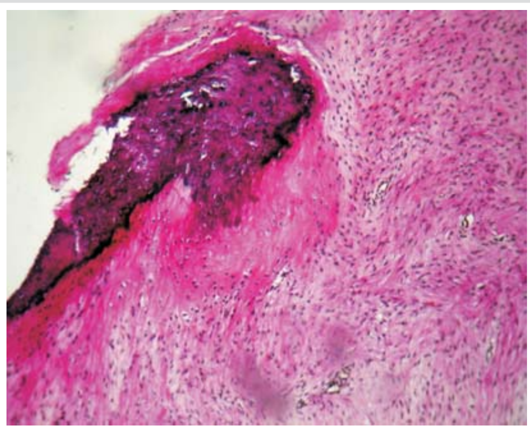

Fig. 22. Aspects at 42 days. Callus with enchondrale ossification. (group II) (Van Gieson, 10X)

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