Is Atrophic Nonunion a Misnomer – A Hospital-based Prospective Cross-Sectional Study

Pseudoartrose atrófica é um termo incorreto? – Um estudo hospitalar transversal prospectivo

Sandeep Kumar Chaudhari[1] Mohit Dhingra[1] Prashant Joshi[2]

[1] Department of Orthopedics, All India Institute of Medical Sciences, Rishikesh, India
[2] Department of Pathology, All India Institute of Medical Sciences, Rishikesh, India

Address for correspondence Mohit Dhingra, MS Orthopedics, Department of Orthopedics, All India Institute of Medical Sciences, Rishikesh, India (e-mail: modisbanu77@gmail.com).

Rev Bras Ortop 2022;57(6):1045–1050.

Abstract

Objective The present study was conducted to estimate histologically the proportion of avascularity of fracture ends in case of nonunion of long bones.

Methods A total of 15 cases of established quiescent nonunion were operated according to the standard protocol and the fracture ends were evaluated histologically. The biopsied tissue was briefly fixed with formalin, embedded with paraffin (FFPE), and 5-micron sections were stained with hematoxylin and eosin according to standard protocols. Immunohistochemistry with anti-CD31 antibody (JC70A clone, DBS) was performed manually using standard protocols.

Results All cases of quiescent nonunion were included; radiologically, 2 cases were oligotrophic, and 13 cases were of atrophic nonunion. A total of 20% of the patients were females, 40% were in the age group between 31and 40 years old, and, radiologically, all cases were of atrophic nonunion. All cases showed positivity for CD-31 on immunohistochemistry. The blood vessel density was category I in 13.33% of the cases and category II in 86.67% of the cases. Four cases presented with mild inflammation and two presented with moderate inflammation. The average vessel count was 10 per high power field in the age groups between 20 and 30, 31 and 40, and 41and 50 years old. The age group between 61 and 70 years old showed an average vessel count of 4 per high power field. The difference in the vessel counts of oligotrophic and atrophic nonunion was not significant. No correlation was observed in the density of vessel count and duration of nonunion

Keywords
- fractures, ununited
- fractures, bone
- antigen, CD31

The present work was developed at the Department of Orthopedics, All India Institute of Medical Sciences, Rishikesh, India

received
December 21, 2021
accepted after revision
February 18, 2022
published online
July 6, 2022

DOI https://doi.org/10.1055/s-0042-1746180.
ISSN 0102-3616.

Thieme Revinter Publicações Ltda., Rua do Matoso 170, Rio de Janeiro, RJ, CEP 20270-135, Brazil

Conclusion The nomenclature for the classification of nonunion into atrophic, oligotrophic, and hypertrophic needs revision. Our findings do not support that atrophic and oligotrophic nonunion are histologically different.

Resumo

Objetivo O presente estudo estimou a proporção de avascularidade histológica das extremidades das fraturas em caso de pseudoartrose de ossos longos.

Métodos No total, 15 casos de pseudoartrose quiescente estabelecida foram operados de acordo com o protocolo padrão e as extremidades da fratura foram avaliadas histologicamente. Em resumo, o tecido biopsiado foi fixado em formalina e embebido em parafina (FFPE); secções de 5 mícrons foram coradas com hematoxilina e eosina de acordo com os protocolos padrões. A imunohistoquímica com anticorpo anti-CD31 (clone JC70A, DBS) foi realizada manualmente segundo protocolos padrões.

Resultados Todos os casos de pseudoartrose quiescente foram incluídos; 2 eram de pseudoartrose oligotrófica e 13 eram de pseudoartrose atrófica à radiologia. Destes, 20% eram de pacientes do sexo feminino, 40% de indivíduos entre 31 e 40 anos de idade e todos os casos eram de pseudoartrose atrófica à radiologia. Todos os casos eram positivos para CD-31 à imunohistoquímica. A densidade dos vasos sanguíneos era de categoria I em 13,33% dos casos e de categoria II em 86,67%. Quatro casos apresentavam inflamação branda e dois apresentavam inflamação moderada. O número médio de vasos era de 10 por campo de alta potência na faixa etária de 20 a 30, de 31 a 40 e de 41 a 50 anos. A faixa etária de 61 a 70 anos apresentava, em média, 4 vasos por campo de alta potência. A diferença nos números de vasos em pseudoarthroses oligotróficas e atróficas não foi significativa. Não houve correlação entre a densidade de vasos e a duração da pseudoartrose.

Conclusão A nomenclatura de classificação da pseudoartrose em atrófica, oligotrófica e hipertrófica precisa ser revista. Nossos achados não indicam que a pseudoartrose atrófica e oligotrófica sejam histologicamente diferentes.

Palavras-chave
- antígeno CD31
- fraturas ósseas
- fraturas não consolidadas

Introduction

The literature has plenty of information on the process of fracture healing.[1] Also, there are studies on the regulations of events that occur during fracture healing. But there is always a lack of consensus and need for further studies. Most fractures unite well, but some do present with complications of delayed union and nonunion. Lower limb fractures account of one-third of all fractures and result in significant morbidity when associated with open injuries and delayed treatment.[2] One of the common morbidities associated with these fractures is nonunion, which accounts for between 5 and 10% of all fractures.[3] Nonunion is described as cessation of any further healing, radiologically, on 3 consecutive x-rays taken in a 1-month interval.[4]

Broadly, these nonunions are described as:

- Infected and noninfected depending on the presence of infection in the fracture ends
- Stiff and mobile depending on the presence of movement at the fracture site[5]
- Hypertrophic, oligotrophic, and atrophic nonunion according to the biological activity observed radiologically[6]

The assessment of biological activity has been predominantly based on radiological parameters and have been followed for a long time. It is a common preconception that hypertrophic nonunions on x-ray are biologically active and bony stabilization is sufficient to attain union. In contrast, atrophic nonunions are considered avascular, acellular, and lack the inherent ability to heal under a correct, stable environment.[6] It has been shown that stability and vascularity of the fracture ends are important factors guiding the callus formation in opposed fractured bones.[7] Although an initial disruption of blood supply may be the cause of nonunion, persistent avascularity may not be a constant factor associated with it.

The aim of the present study is to investigate the histology and find out the incidence of vascularity of the fracture ends of various nonunions.

Materials and Methods

The present study was conducted after obtaining approval from the institutional ethics committee.

Only patients who sustained open fractures with bone loss due to high velocity trauma such as road traffic accident,

fall from height, and gunshot injuries and were infected at some point but now have healed and become quiescent (nondraining) were included in the present study.[8]

The eligibility criteria for the present study were skeletally mature patients presenting with quiescent (nondischarging) nonunion of long bones. All patient with suspected pathological fracture, patients with discharging nonunions of long bones, and patients in the pediatric age group were excluded from the study.

Methodology: All patients fulfilling the inclusion criteria were informed about the methodology of the present study. Informed and written consent were obtained from all patients. All patients had their history taken and were submitted to clinical examination. X rays of each patient were taken and nonunions of long bones were classified according to x ray findings by two independent orthopedic surgeons. The classification was made according to the morphology of the fracture ends and the amount of callus formation.[9] During the operation, intraoperative biopsy samples were taken from the nonunion site (►**Figure 1**).

Histological Examination: Biopsy samples taken from the nonunion site were fixed with 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E); 5-µm sections stained with H&E were analyzed by light microscopy to see the general morphology and the characteristics of the tissue. CD31 immunohistochemistry was employed to highlight the vasculature (►**Figure 2**).

The inflammatory infiltrate comprised of lymphocytes and neutrophils was graded as:

Mild: Inflammatory cells in $<33\%$ of the area of the tissue section

Moderate: Inflammatory cells in between 33 and 66% of the area of the tissue section

Severe: Inflammatory cells in $>66\%$ of the area of the tissue section

The distribution of the blood vessels was semiquantitatively graded on 100x magnification according to the following scheme:[10]

Category 0: No positively stained blood vessels present

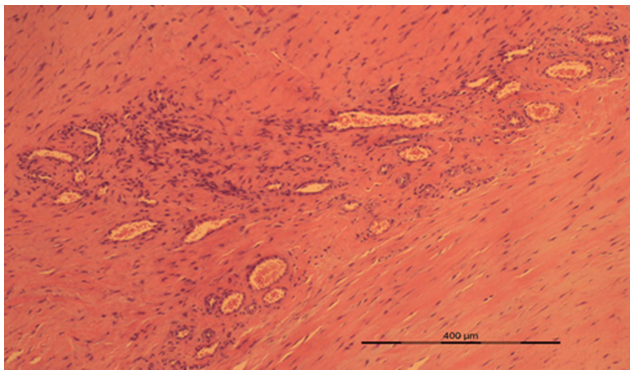

Fig. 2 Histopathological specimen of a patient with atrophic nonunion radiologically showing blood vessel density (category 2) at 400x.

Category 1: Between 1 and 50'% of the field containing blood vessels

Category 2: >50'% of the field containing blood vessels

The density of the blood vessels was quantified by taking the average of 3 noncontiguous 400x fields with the highest blood vessel counts. Each 400x field in the microscope model used corresponds to 0.55 mm^2.[10]

Results with Statistical Analysis

In our series of 15 cases, the mean age of the patients was 40.60 ± 12.99 years old. Most patients were in the age group between 31 and 40 years old (40%). Our series had 12 males and 3 females. The cases of nonunion were in the tibia (66.7%), the femur (13.3%), the forearm bones (13.3%), and the humerus (6.7%). Thirteen cases were atrophic and 2 were oligotrophic.

The duration of nonunion was 16.87 ± 6.59 months. All cases were positive for CD31 (100%) (►**Figure 3**). The average vessel count was 9.27 ± 4.28. Two cases had blood vessel density in category 1, and the rest were in category 2 (►**Figure 4**). Most cases did not show any inflammation (60%), with mild inflammation in 4 cases (►**Table 1**).

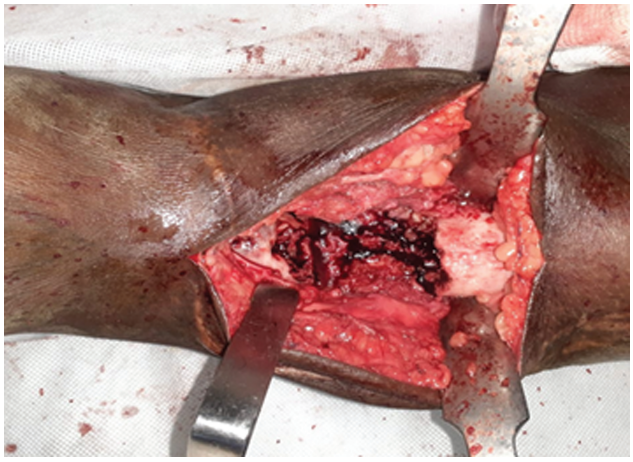

Fig. 1 Atrophic nonunion site (intraoperative image).

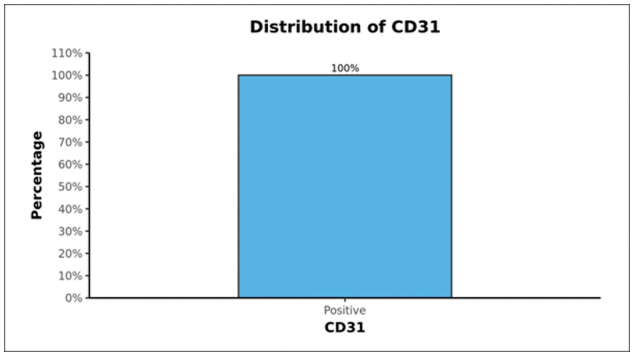

Fig. 3 Distribution of the participants in terms of CD31 ($n = 15$). 100.0% of the participants were CD31-positive; 0.0% of the participants were CD31-negative.

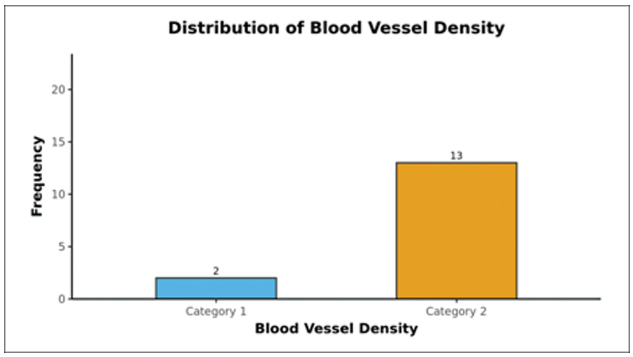

Fig. 4 Distribution of the participants in terms of blood vessel density ($n = 15$).

There was a moderate negative correlation between average vessel count and age (years old), and this correlation was not statistically significant (rho = −0.4; $p = 0.145$) (►**Figure 5** and ►**Table 2**).

There was a weak negative correlation between duration of nonunion (months) and age (years old), and this correlation was not statistically significant (rho = −0.11; $p = 0.691$) (►**Figure 6** and ►**Table 3**).

On correlating the radiological features with the histological features of the types of nonunion, it was seen that all cases that were radiologically atrophic were histologically hypertrophic. They had good vessel count and density. The two cases that were oligotrophic on x-ray were oligotrophic histologically (►**Figure 7**).

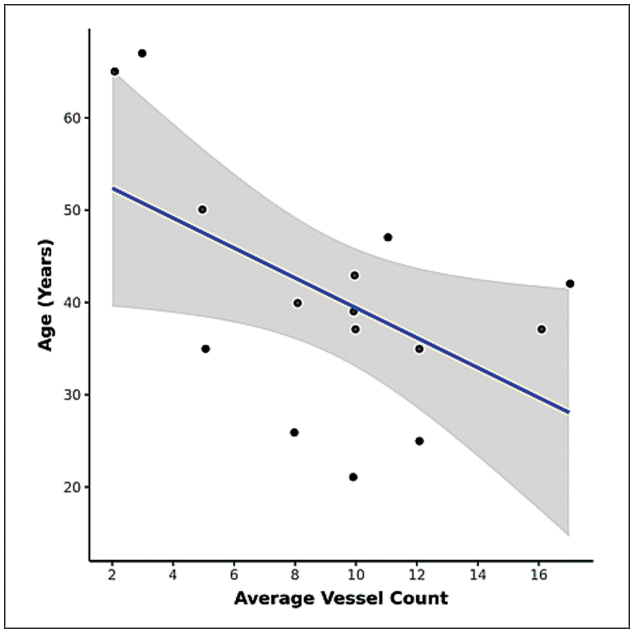

Fig. 5 Correlation between average vessel count and age (years old) ($n = 15$). The above scatterplot depicts the correlation between average vessel count and age (years old). Individual points represent individual cases. The blue trendline represents the general trend of correlation between the two variables. The shaded grey area represents the 95% confidence interval of this trendline.

Table 1 Details of all patients with related parameters

All Parameters	Mean ± SD || Median (IQR) || Min-Max || Frequency (%)
Age (years old)	40.60 ± 12.99 || 39.00 (35.00–45.00) || 21.00–67.00
Age	
21–30	3 (20.0%)
31–40	6 (40.0%)
41–50	4 (26.7%)
61–70	2 (13.3%)
Gender	
Male	12 (80.0%)
Female	3 (20.0%)
Nonunion site	
Tibia	10 (66.7%)
Femur	2 (13.3%)
Radius/Ulna	2 (13.3%)
Humerus	1 (6.7%)
Type of nonunion	
Atrophic	13 (86.7%)
Oligotrophic	2 (13.3%)
Duration of nonunion (months)	16.87 ± 6.59 || 16.00 (11.50–19.50) || 9.00–33.00
CD31 (positive)	15 (100.0%)
Blood vessel density	
Category 1	2 (13.3%)
Category 2	13 (86.7%)
Average vessel count	9.27 ± 4.28 || 10.00 (6.50–11.50) || 2.00–17.00
Inflammation	
None	9 (60.0%)
Mild	4 (26.7%)
Moderate	2 (13.3%)

Abbreviations: IQR, interquartile range, Max, maximum ; Min, minimum; SD, standard deviation.
The variable duration of **nonunion** (months) was normally distributed (Shapiro-Wilk test: $p = 0.157$).

Discussion

Nonunion of long bone following fracture is a common complication, especially when associated with open injuries and bone loss, leading to high morbidity and clinical burden. The US Federal Drug Administration council defines nonunion as "failure to achieve union by 9 months since the injury, and for which there has been no clinical and radiological signs of healing for 3 consecutive months".[11] Traditionally, x-rays have been used to assess the biological activity in the nonunion fracture site on the basis of which nonunion is classified in three groups hypertrophic,

Table 2 Correlation between blood vessel count and age of the patients

Correlation	Spearman correlation coefficient	*p-value*
Average vessel count versus age (years old)	−0.4	0.145

Nonparametric tests (Spearman correlation) were used to explore the correlation between the two variables, as at least one of the variables was not normally distributed.
There was a moderate negative correlation between average vessel count and age (years old), which was not statistically significant (rho = −0.4; $p = 0.145$).

Table 3 Correlation between duration of nonunion and age of the patients

Correlation	Spearman correlation coefficient	*p-value*
Duration of nonunion (months) versus age (years old)	−0.1	0.691

Nonparametric tests (Spearman correlation) were used to explore the correlation between the two variables, as at least one of the variables was not normally distributed.
There was a weak negative correlation between duration of nonunion (months) and age (years old), which was not statistically significant (rho = −0.11; $p = 0.691$).

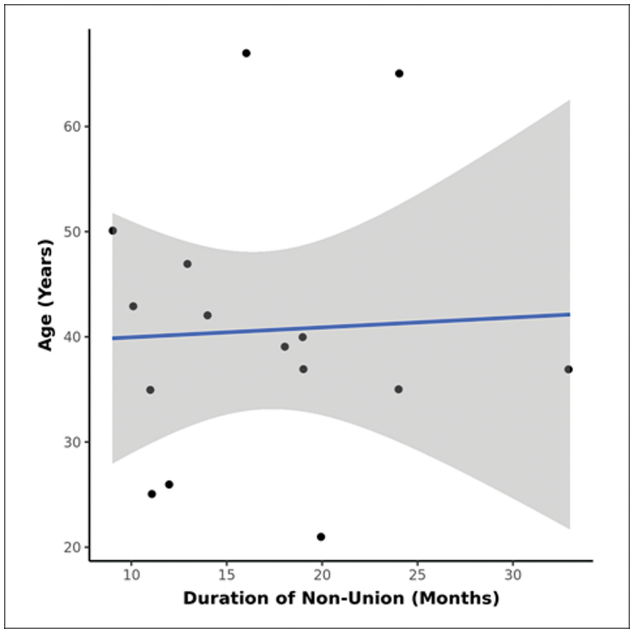

Fig. 6 Correlation between duration of nonunion (months) and age (years old) ($n = 15$). The below scatterplot depicts the correlation between duration of nonunion (months) and age (years old). Individual points represent individual cases. The blue trendline represents the general trend of correlation between the two variables. The shaded grey area represents the 95% confidence interval of this trendline.

oligotrophic, and atrophic nonunion. It is commonly believed that hypertrophic nonunions are biologically active and vascular and can heal if a correct, stable environment is provided. In contrast, atrophic nonunions are considered to be relatively avascular, acellular and inert, and have less potential to heal even under the correct stable environment.[7,12]

Since there has been a shift in research from the anatomical level to the molecular level, the present study was conducted to define the molecular characteristics of the nonunion site and its correlation with radiological and anatomical parameters.

In the present study, 15 patients with clinically healed quiescent nonunion were included. The mean age (years old) was 40.60 ± 12.99; 12 (80.0%) of the participants were male and 3 (20.0) were female. One similar previous study by Reed et al.[12] included 22 patients with a mean age of 47.18 years old, 17 male and 5 females. In another in vitro study conducted by Vallim et al.,[13] 15 patients with a mean age of 46.4 years old with atrophic nonunion were included, 9 males and 6 females.

In our study, we had 13 (86.7%) cases of atrophic nonunion and 2 (13.3%) cases of oligotrophic nonunion classified radiologically. The mean duration of nonunion (months) was 16.87 ± 6.59. In the study by Reed et al.,[12] 11 out of 22 patients had hypertrophic nonunions (median time after fracture: 21 months)) and 11 patients had atrophic nonunions (median time after fracture: 24 months). In the study by Vallium et al.,[13] only cases of atrophic nonunion were included.

In our study, the histology of all 15 (100.0%) patients were found to be CD31-positive. The mean average vessel count was 9.27 ± 4.28. Nine of the patients had no inflammation, 4 (26.7%) had mild inflammation, and 2 (13.3%) had moderate inflammation. In another study by Reed et al.,[12] all of the cases stained for CD 31 were found to be positive and were not different histologically; however, a difference between the densities of the vessels of the different types of nonunion was observed, but it was not significant ($p > 0.05$). In the study by Brownlow et al.,[14] done on rabbits, it was found that there was a significant difference ($p < 0.05$) between the control and experimental groups when the rabbits were sacrificed at 1 week, but at the end of 8 weeks and 16 weeks, there was no significant difference in the vessel density of the nonunion site of both groups. Another in vitro study by Vallim et al.[13] on atrophic nonunion stromal cells (NUSCs) showed that NUSC, bone marrow stromal cells (BMSCs), and osteoblasts required equal time for the cell population to double (NUSCs average: 7.8 ± 3.8 days; BMSCs average: 5.4 ± 1.8 days; and osteoblasts average: 9.0 ± 5.1 days) and there was no statistically significant difference between the 3 groups. It was also found that the b-galactosidase activity in NUSC cultures was similar to that observed in BMSCs and osteoblasts, suggesting that NUSCs could sustain proliferation to the same extent as BMSCs and osteoblasts.

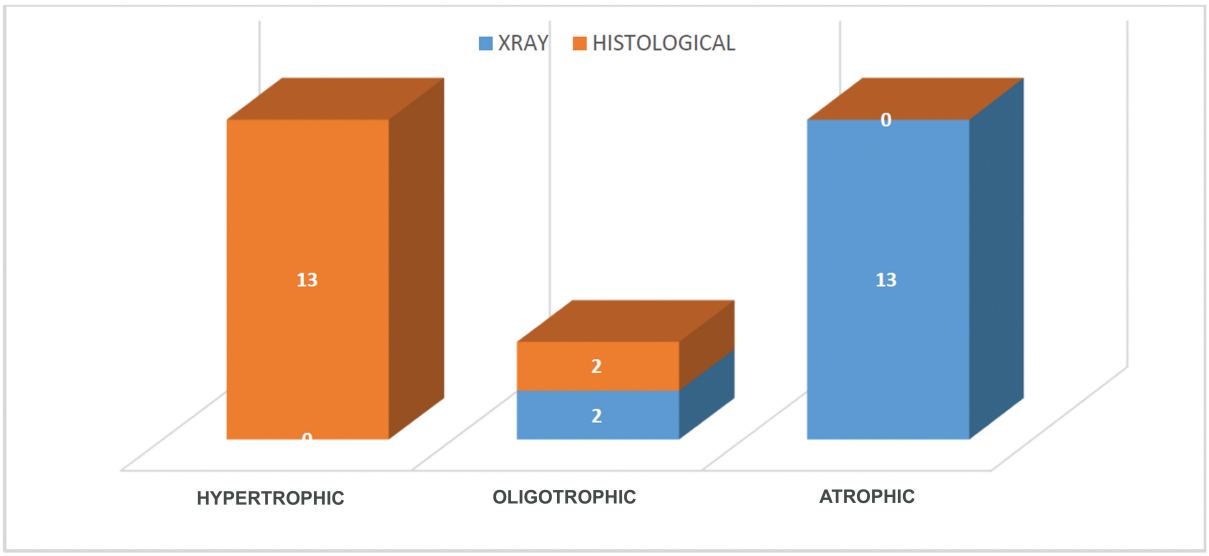

Fig. 7 Comparison of nonunion (radiological versus histological).

Conclusion

The radiological distinction of nonunions into atrophic, oligotrophic, and hypertrophic has been done for a long time. But there is not much histological difference between atrophic and oligotrophic nonunions in terms of vessel density and activity. Atrophic nonunion, as the name suggests, is not hypo- or avascular. It still has the potential of new bone formation if provided with adequate stabilization and chemotactic factors.

Financial Support

No funding was received from any agency for the present study.

Conflict of Interests

The authors have no conflict of interests to declare.

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