Journal of Orthopaedic Surgery 2005;13(1):3-7

Histomorphometric changes in the vessel wall at the site of amputation in diabetic patients—do they influence healing of the stump?

SVLG Naidu, S Sengupta
Department of Orthopaedic Surgery, University of Malaya Medical Center, Kuala Lumpur, Malaysia

ABSTRACT

Purpose. To predict healing of the stump by assessing the microscopic vascular changes at the amputation site.
Methods. A cohort study was conducted on 39 patients, 18 of them had below-knee amputation (group A) and 21 had ray amputation of a single toe (group B). Biopsies were taken from the anterior and posterior tibial arteries and the venae comitantes of group A patients. For group B patients, biopsies of the digital artery and dorsal vein of the toe were taken.
Results. In group A, 15 patients required no further amputation (group A1) and 3 underwent a further above-knee amputation (group A2). In group B, 16 required no further amputation (group B1) and 5 underwent a below-knee amputation (group B2). Lumen narrowing caused by intimal thickening of the arteries was significantly different between groups A1 and A2 ($p<0.05$). Lumen narrowing of the dorsal veins between groups B1 and B2 was also significantly different ($p<0.05$). The proportion of the vessel walls made up of intima and media was significantly different in both A1 and A2 as well as B1 and B2 groups. The proportion of total wall thickness over the total diameter of the vessel was not significantly different between both subgroups of A and B.
Conclusion. Intimal thickening and medial thinning in the arteries can be used to predict the stump healing in patients who underwent below-knee amputation. For ray amputation patients, similar changes occurred in the dorsal veins, and this finding can also be used to predict the healing of the stump. However, intimal thickening occurred at the expense of the media; therefore, there is little change in the wall thickness.

Key words: *amputation stumps; wound healing*

Address correspondence and reprint requests to: Dr Suguna Venkataramulu LG Naidu, Department of Orthopaedic Surgery, Seremban Hospital, Off Jalan Rasah, 70300 Seremban, Negeri Sembilan, Malaysia. E-mail: suguvlgn@hotmail.com

INTRODUCTION

Amputation is one of the methods to treat foot ulcer and gangrene in diabetics. The extent of amputation depends on the severity of the lesion, and the decision to amputate is based on the clinical judgement of the surgeon. However, some unsuccessful cases of amputation may require reamputation at a higher level. The aim of this study was to determine whether the quantitative changes of the lumen, intima, and media could predict the healing of the stump. Hence, the microscopic vascular changes at the amputation site were assessed.

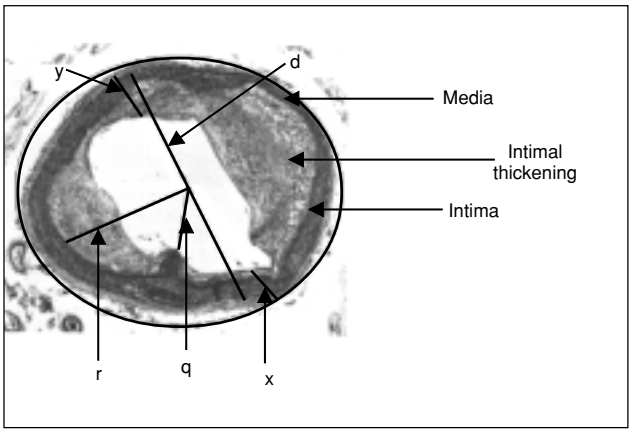

Figure Diagrammatic description of a sample of the vessel and the measurements: percentage of lumen occlusion= $[(\pi r^2-\pi q^2)/\pi r^2]x100\%$; the thickness contributed by the vessel wall=$[(x+y)/d]x100\%$. Using the linear calibrations on the microscope, the value of the intimal thickness and the medial thickness was obtained and the proportion calculated.

MATERIALS AND METHODS

Between 1 February 2001 and 31 July 2001, 39 diabetic patients (18 women and 21 men), aged 36 to 85 years, underwent amputation of their lower limbs at the University of Malaya Medical Center, Kuala Lumpur, Malaysia. The patients had diabetes for a duration ranging from one to 20 years.

A cohort study was conducted and the patients were divided into 2 groups. Group A patients underwent below-knee amputation for extensive gangrene or ulceration of the foot proximal to the metacarpophalangeal joint up to the level of the distal part of the leg. Group B patients, who had ulcers or dry gangrene of a single toe, underwent ray amputation to salvage the rest of the foot. Group A patients were further classified into groups A1 (no further amputation) and A2 (above-knee amputation); group B patients were further classified into groups B1 (no further amputation) and B2 (below-knee amputation). All the patients were treated with sulbactam-ampicillin preoperatively, and their sugar and electrolyte levels were optimised. Below-knee amputations were performed 15 cm below the medial joint line of the knee without the use of a tourniquet. A posterior flap was used to cover the wound after adequate haemostasis. All patients had their wounds closed with 3/0 nylon sutures. Samples of the anterior and posterior tibial arteries and venae comitantes proximal to the amputation site were taken from the group A patients. Ray amputations were performed by complete excision of the affected toe and its corresponding metatarsal sparing the base. Adequate haemostasis was achieved, and the wound was left open. Samples of the digital artery and dorsal vein of the affected toe at the level of the metatarsophalangeal joint were taken from the group B patients.

The condition of the amputated limbs was reviewed to determine whether a further amputation would be necessary during the same admission or within the next 6 months. Indications for further amputation at a higher level were wound breakdown with extensive tissue damage, wound infection that was not responding to antibiotic therapy, and persistent or progressive gangrene.

The specimens were processed and stained using haematoxylin and eosin and viewed at a magnification of 4.5. Diagrammatic description of the calculations is shown in the Figure, and the characteristics of the vessels were noted: narrowing of the blood vessel caused by intimal thickening was measured as a percentage of occluded lumen area to the original lumen area. This percentage was divided into 4 grades: grade 1, 0%–25%; grade 2, 26%–50%; grade 3, 51%–75%; and grade 4, 76%–100%. The original area of the lumen was presumed to stretch to the media because the normal intimal thickness was negligible. The proportion of wall thickness contributed by the intima and media was also expressed as a percentage. The extent of thickening of the vessel wall was measured as the total wall thickness over the total diameter of the vessel and again expressed as a percentage.

Measurements were made using calibrations in the microscope in terms of 'units'. The radius of the lumen and the area (πr^2) were determined. If the radius was not uniform, then the mean radius was calculated by averaging the largest and smallest radii. All measurements were converted to percentages

Table 1
Analysis of the percentage of lumen occlusion of the vessels and the outcome of an initial below-knee amputation in group A using the unpaired *t*-test

	Anterior tibial artery		Anterior venae comitantes		Posterior tibial artery		Posterior venae comitantes	
Groups	A1	A2	A1	A2	A1	A2	A1	A2
No. of patients	15	3	15	3	15	3	15	3
Percentage (%)	83	17	83	17	83	17	83	17
Range (%)	29–51	81–89	0–18	9–23	11–49	82–92	0–13	10–20
p value	0		0.14		0		0.19	
Significance	Yes		No		Yes		No	

to eliminate anatomic differences due to age, sex, and build of the patients. The tabulated data were analysed using the Statistical Package for the Social Science (Version 5.0; SPSS Inc, Chicago [IL], US) and using the statistics guidebook.[1,2] A p value of less than 0.05 was taken as statistically significant for all the tests (unpaired *t*-tests). All the measurements were performed in duplicate manually and independently. Any values that were not consistent were subjected to repeated assessment until the values were found to be in agreement.

Table 2
Analysis of the percentage of lumen occlusion of the vessels and the outcome of an initial ray amputation in group B using the unpaired *t*-test

	Digital artery		Dorsal vein	
Groups	B1	B2	B1	B2
No. of patients	16	5	16	5
Percentage (%)	76	24	76	24
Range (%)	20–95	75–90	0–9	11–16
p value	0.08		0	
Significance	No		Yes	

RESULTS

In group A, 15 patients required no further amputation (group A1) and 3 underwent an above-knee amputation (group A2). In group B, 16 required no further amputation (group B1) and 5 underwent a below-knee amputation (group B2). The risk of above-knee amputation after a below-knee amputation was 17% and the risk of below-knee amputation after an initial ray amputation was 24%.

The percentage results of the lumen occlusion caused by intimal thickening of groups A and B are shown in Tables 1 and 2, respectively. The proportions of media and intima contributing to wall thickness between groups A1 and A2 as well as between groups B1 and B2 were significant ($p<0.05$). The percentage of thickness of the wall over the whole diameter of the vessel between groups A1 and A2 was not significant for the values of anterior tibial artery, anterior venae comitantes, posterior tibial artery, and posterior venae comitantes. The percentage of thickness of the wall over the whole diameter of the vessel between groups B1 and B2 was not significant for the values of the digital artery and dorsal vein.

DISCUSSION

In this study, the risk of an above-knee amputation was found to be 17% in those having a primary below-knee amputation. The risk for primary ray amputees to have a below-knee amputation was 24%. Similar findings were reported by Ebskov and Josephsen[3] in 1980. They found that 19% of diabetic amputees would undergo reamputation within a 6-month period.

Ray amputation has a higher incidence of reamputation because infrapopliteal vessels are mainly affected in diabetic patients. In considering the vascular aspects, the toes are furthest from the arterial trifurcation at the leg and are supplied by the distal end of the infrapopliteal vessels, which is involved in atherosclerotic occlusions. The level of below-knee amputation was 15 cm below the knee joint, which is mainly supplied by the popliteal vessels and the proximal level of its branches.

The minimum percentage of intimal thickening was 20% and 28% for the anterior and posterior tibial arteries, respectively, whereas the maximum percentage was 89% and 92%, respectively. This finding shows that the leg arteries of the diabetic patients are affected by at least 20% of intimal thickening. Patients in group A2 had the thickness of both arteries in the fourth quartile range, whereas those in group A1 had intimal thickening in the first quartile range. The difference between the 2 subgroups was statistically significant ($p<0.05$).

There was a small amount of intimal thickening in the anterior and posterior venae comitantes in the group A patients. This showed that there was considerable venous involvement in atherosclerosis, but not as much as in the arteries. The difference between group A1 and A2 was not statistically significant.

The patients who underwent an initial ray amputation did not show a similar pattern of vessel occlusion. Group B2 patients showed a smaller range of variation in arterial intimal thickening (75%–95%) compared to group B1 (20%–95%) but the difference was not statistically significant. The level of dorsal vein occlusion showed a significant difference between the 2 subgroups ($p<0.05$), but the percentage of dorsal vein occlusion was in the 0% to 20% range, indicating that the level of occlusion caused by intimal thickening was small.

The results of the outcome of the ray amputation suggested that some other factors determined the pathology of the foot. A likely but controversial explanation is the presence of a microvascular disease in the foot. LoGerfo and Coffman[4] disagreed with this explanation, because they found that the lesions in the foot vessels of diabetic and non-diabetic patients were similar. However, Ferguson et al.[5] found that such a microvascular disease was present, because the histological sections of the foot vessels had the appearance of capillary basement membrane thickening (ie abnormal proliferation of endothelial and smooth muscle cells). Whatever the status of the medium-sized vessels in the foot, if the small vessels are not patent and functioning, the tissue is likely to be ischaemic and may turn gangrenous.[6–8]

The dorsal vein of the toes is a predictor of the outcome of an initial ray amputation because these veins lie in close proximity to the toe and lie under the dermis where they develop the inflammatory reaction. However, the degree of changes seen in the dorsal veins is small (0%–16%), indicating that vascular obstructive disease does not affect the veins as much as in the arteries (20%–95% occlusion). Normally, the intima is one-cell thick with one thin layer of basement membrane under it and a much thicker media. In diabetic patients, the intima undergoes thickening, and the media undergoes medial calcification. However, the media does not thicken much because the calcification replaces the normal tissue, which causes the vessels to become more rigid and less distensible.

The medial layer of both the arteries in the group A2 patients contributed less to the total wall thickness compared with the medial layer of the group A1 patients. The veins show similar changes. The intimal thickening was considerable enough to cause a change in the quantitative aspect of the media. The differences in the outcome of the 2 subgroups of A show that the ratio of intimal thickness and the medial thickness, which contributes to the total wall thickness, is statistically significant. We therefore conclude that the intimal thickening has occurred at the expense of the media.

For the patients who underwent an initial ray amputation, there were significant differences in the proportion of the intima and media of digital artery in both the B1 and B2 groups. This finding is probably because the arteries are in close proximity to the diseased toe and the inflammation causes changes in the intima rather than the media. The changes in the thickness of the intima and the media in the dorsal vein were similar. The changes were found to be statistically significant for the intima and media of the arteries and veins.

The total thickness of the vessel wall contributing to the total vessel diameter shows no obvious difference between groups A1 and A2 or groups B1 and B2. Although there were changes seen in the intimal and medial thickness of the vessel wall, these changes were very small compared with the total diameter of the vessel.

CONCLUSION

Our study shows that histopathological changes in the arterial walls can be used to predict the healing of the stump in patients who underwent below-knee amputation. The arterial lumen occlusion is higher and the media becomes thinned out due to the intimal thickening, if the outcome is an above-knee amputation within a 6-month period. The veins are less useful for predicting the healing of a below-knee amputation stump. As for ray amputations, the changes in the dorsal veins can predict healing of the stump. However, very little difference occurs in the dorsal veins in the 2 groups B1 and B2 to be of predictive value.

REFERENCES

1. Statistical package for social sciences. In: Bowman KJ, Cahill M, editors. New York: McGraw Hill; 1998.

2. Bland M. An introduction to medical statistics. Oxford: Oxford University Press; 1999.
3. Ebskov B, Josephsen P. Incidence of reamputation and death after gangrene of the lower extremity. Prosthet Orthot Int 1980; 4:77–80.
4. LoGerfo FW, Coffman JD. Current concepts. Vascular and microvascular disease of the foot in diabetes. Implications for foot care. N Engl J Med 1984;311:1615–9.
5. Ferguson MW, Herrick SE, Spencer MJ, Shaw JE, Boulton AJ, Sloan P. The histology of diabetic foot ulcers. Diabet Med 1996;13(Suppl 1):S30–3.
6. Tooke JE, Brash PD. Microvascular aspects of diabetic foot disease. Diabet Med 1996;13(Suppl 1):S26–9.
7. Kastrup J, Lassen NA, Parving HH. Diabetic microangiopathy: a factor enhancing the functional significance of peripheral occlusive arteriosclerotic disease. Clin Physiol 1984;4:367–9.
8. Beach KW, Strandness DE Jr. Arteriosclerosis obliterans and associated risk factors in insulin-dependent and non-insulin-dependent diabetes. Diabetes 1980;29:882–8.