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Lower limb arterio-venous shunts, autonomic neuropathy and diabetic foot

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Summary

We have quantitatively assessed the percentage of lower limb arterio-venous (a-v) shunting using a radioisotopic technique and correlated it with autonomic neuropathy evaluated by cardiovascular tests. We have studied three groups of diabetic patients: Group A, 12 non-neuropathic subjects without foot lesions; Group B, 12 neuropathic subjects without foot lesions; Group C, 12 neuropathic subjects with recurrent foot ulcers. Shunting was higher in Group C (10.4 ± 2.7%) than in Group B (6.8 ± 2.3%, $P < 0.01$) and Group A (3.8 ± 1.2%, $P < 0.001$). Shunts in Group B were higher than in Group A ($P < 0.05$). All the tests exploring autonomic function were more impaired in Groups B and C than in Group A, with no difference between Groups B and C. A direct correlation was found between a-v shunting and the following cardiovascular tests: postural hypotension (PH) ($r = 0.41$, $P < 0.02$), sustained handgrip (SH) ($r = 0.56$, $P < 0.001$), deep breathing (DB) ($r = 0.40$, $P < 0.005$) and lying to standing (LS) ($r = 0.44$, $P < 0.01$). A positive correlation was also found between a-v shunts and duration of the disease ($r = 0.62$, $P < 0.001$). Arterio-venous shunting was found to be directly related to autonomic neuropathy even if the higher shunting found in the patients with foot ulcers was not related to a higher degree of autonomic involvement; in addition, this group of patients was characterized by having a more advanced sensory and motor neuropathy. In conclusion, autonomic neuropathy, through its influence on a-v shunts, may play a role in the pathogenesis of diabetic foot, but peripheral neuropathy probably plays a key role in conditioning the development of the overt clinical manifestations of diabetic foot.

Key words: Autonomic neuropathy; Diabetic foot; Arterio-venous shunts

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Introduction

The clinical entity which goes under the name of diabetic foot includes lesions of ischaemic and/or neuropathic origin such as ulcers, gangrene, bone demineralization and joint deterioration [1]. In about 30% of cases, neuropathic involvement of motor, sensory and autonomic nerves is clearly predominant [2–4]. Indirect evidence has shown that a sympathetic impairment in diabetic foot may be responsible for changes in peripheral blood flow distribution, which is probably involved in the pathogenesis of such a lesion. In fact, diabetic foot shows a decreased femoral arterio-venous (a-v) oxygen difference [5] and reduced peripheral resistance during Doppler registration [6]. These data have been interpreted to be a result of the increased blood flow through a-v shunts, which are tonically closed by the sympathetic nervous fibers, but are abnormally dilated in the presence of diabetic neuropathy. The aim of our work was to evaluate the percentage of a-v shunting in diabetic subjects with and without neuropathy and/or recurrent neuropathic ulcers and to correlate it with autonomic neuropathy as assessed by cardiovascular autonomic tests.

Materials and Methods

Patients

This study was approved by the Ethical Committee and informed consent was obtained from all diabetic patients. Thirty-six male diabetic patients were admitted to the study after a thorough interview to eliminate any potential interfering neurological condition (alcohol addiction, thyroid disease, lumbar root disease, etc.). Their clinical characteristics are given in Table 1. Following the recommendations of the San Antonio Consensus Conference on Diabetic Neuropathy [7] our patients were distributed into Class I (absence of demonstrable symptoms or signs of neuropathy) and Class II (presence of symptoms and/or signs of neuropathy). A further distinction was made among Class II patients depending on the presence of foot lesions. Thus the patients were divided into the following three groups: 12 diabetic Class I patients (Group A); 12 diabetic Class II patients without foot lesions (Group B); and 12 diabetic Class II patients with recurrent neuropathic ulcers and active lesions at the time of the examination (Group C). There were no significant differences in mean age and duration of diabetes among the three groups.

As required by the Consensus Conference 'Our Validated Questionnaire', we used the Neurological Symptom Score and Neurological Disability Score proposed by Dyck [8].

TABLE 1

Characteristics of the patients included in the study. Group A (n = 12), diabetic patients without neuropathy and foot lesions; Group B (n = 12), diabetic patients with neuropathy, without foot lesions; Group C (n = 12), diabetic patients with neuropathy and neuropathic foot ulcers.

	Group		
	A	B	C
Age (years)	50.6 ± 6.4	53.2 ± 11	51 ± 13
Diabetes (years)	15.5 ± 1.6	16.6 ± 6.8	16.2 ± 4.3
Type 1/2	5/7	6/6	8/4
FBG (mmol/l)	6.5 ± 3.6	8.6 ± 1.8	7.5 ± 3.2
HbA1 (%)	11.2 ± 2.3	9.9 ± 3.1	10.3 ± 1.5
Retinopathy (N/B/P)	4/5/3	3/4/5	2/7/3
Albuminuria (N/M)	8/4	8/4	7/5
Creatinine (mmol/l)	85.1 ± 13.3	106.1 ± 18.6	99.8 ± 16.8

Retinopathy: N, absent; B, background retinopathy; P, proliferative retinopathy.
Albuminuria: N, absent; M, presence of macroalbuminuria.
FBG, fasting blood glucose.

Cardiovascular test of autonomic function
All diabetic subjects underwent the same tests [9–11].

(1) *Valsalva ratio (VR).* ECG measurement of the heart rate was performed during 15 s of forced expiration and subsequently during 30 s of complete relaxation. The VR was calculated as the ratio between the longest R–R interval during the relaxation period and the shortest R–R interval during forced expiration [12].

(2) *Lying to standing (LS).* The increase in heart rate following the shift from clinostatism to orthostatism starts approximately 15 beats after standing up and decreases at about the 30th beat. The ratio between the longest and the shortest R–R interval was measured [13].

(3) *Deep breathing (DB).* The subject breathed deeply at 6 breaths/min. The difference between maximal and minimal heart rate during each breathing cycle was measured [14].

(4) *Postural hypotension (PH).* The difference between lying and standing systolic blood pressure was measured, recording the blood pressure for 5 min after standing up [15].

(5) *Sustained handgrip (SH).* The variation in diastolic blood pressure was recorded as the patient kept his hand in a grasping position at 30% of maximal voluntary contraction for up to 5 min. The difference between diastolic blood pressure just before release of handgrip and before starting was measured [16].

Radioisotopic study of the a-v shunts
The percentage of a-v shunting was obtained by a simplified radionuclide technique derived from the work of Lopez-Majano et al. [17] and Rhodes et al. [18]. This technique is based on the principle that after intra-arterial injection of labelled albumin microspheres with a diameter larger than the calibre of the peripheral capillaries, the particles are totally trapped in the capillary bed; therefore, if a-v shunting is present, some particles enter the venous circulation and are trapped in the pulmonary capillaries. Consequently, the radioactivity detected in the lungs is directly related to the degree of a-v shunting. In the present study we used human serum albumin microspheres obtained from a commercial radiopharmaceutical kit (TCK, Sorin Biomedica, Soluggia, Italy) labelled with ^{99m}Tc; 95% of the particles had a diameter ranging from 7 to 25 microns.

Early in the morning after a rest period of half an hour in a room at standard temperature, the patients underwent the radioisotopic study. A 3-mCi dose of ^{99m}Tc microspheres was injected into the femoral artery of each patient; in Group C patients we chose a leg with foot ulcers. After 5 min, two lung scans were performed in anterior and posterior views by a LFOW gamma-camera connected to a minicomputer. Another 3-mCi dose (carefully prepared to be equal to the first and checked by a dose calibrator) was subsequently injected into an arm vein and anterior and posterior lung scans were again performed. Lung scan duration was 3 min. A percent index of a-v shunting (%a-v) was calculated by the following equation: $\%\text{a-v} = a \times 100/(b - a)$, where a is the geometric mean of the lung scan counts recorded after arterial injection; b the geometric mean of the lung scan counts recorded after venous injection; and b-a the lung scan counts relative to a hypothetical 100% a-v shunting. No side effects were recorded during and after the test.

Nerve conduction velocity
Nerve conduction studies were performed with standard surface stimulating and recording techniques on the peroneal motor and sural sensory nerves, with standard technique described elsewhere [19].

Vibratory perception threshold
Vibratory sensitivity was measured with a Biothesiometer (Biomedical Instrument Co., Newbury, Ohio) by detecting the vibratory perception threshold. Measurements with the biothesiometer were performed on the right side at the hallux and external malleolus according to the method described previously [20].

Fasting plasma glucose (glucose oxidation method), glycosylated haemoglobin (HbA1) [microcolumn method of a commercial kit (Boehringer, Mannheim, F.R.G.)], serum creatinine, albuminuria (albustix AMES) and blood pressure were recorded the day of the study. An eye examination test had been performed not more than 3 months before. In order to exclude significant peripheral vascular disease, the patients were evaluated by Doppler ultrasound technique and only those showing an ankle index pressure > 1.0 in the absence of major vascular calcifications were included in the study.

Statistics

Results are expressed as mean ± standard deviation. Statistical analysis was performed using analysis of variance (ANOVA). Spearman's rank correlation coefficients were calculated. Significance was accepted at the 5% level.

Results

Table 2 shows the results of the cardiovascular autonomic tests for the three groups of patients (Groups A, B and C). While all the cardiovascular tests were found to be more compromised in Groups B and C than Group A, none of these tests was able to differentiate Group B from Group C.

The percent index of a-v shunting was higher in Group C (10.4 ± 2.7%) than in Group B (6.8 ± 2.3%, $P < 0.01$) and Group A (3.8 ± 1.2%, $P < 0.001$). It was higher in Group B than in Group A ($P < 0.05$) (Fig. 1).

TABLE 2

Response to the cardiovascular autonomic tests. Results of the five tests used to evaluate autonomic neuropathy in the three groups (see Table 1). VR, Valsava ratio; DB, deep breathing; LS, lying to standing; PH, postural hypotension; SH, sustained handgrip. (The results are expressed as mean ± SD. A vs B, [a]$P < 0.05$; [b]$P < 0.01$. A vs C, [c]$P < 0.05$; [d]$P < 0.01$; [e]$P < 0.001$).

	VR[b,d]	DB[a,c]	LS[a,c]	PH[b,c]	SH[a,d]
A	1.36 ± 1.9	17 ± 4	1.07 ± 0.02	7 ± 5	22 ± 5
B	1.17 ± 0.12	12 ± 5	1.03 ± 0.03	18 ± 9	13 ± 9
C	1.19 ± 0.3	11 ± 5	1.04 ± 0.07	22 ± 8	10 ± 7

In order to detect any relationship between a-v shunting and autonomic neuropathy, the percentage of a-v shunts of all 36 subjects, as measured by the radioisotopic studies, was correlated with each one of the cardiovascular tests. A positive correlation was found with PH (r = 0.41, $P < 0.02$), SH (r = 0.56, $P < 0.001$), DB (r = 0.40, $P < 0.05$) and LS (r = 0.44, $P < 0.01$). Also, the duration of the disease was related to the percentage of a-v shunts (r = 0.62, $P < 0.001$).

The nerve conduction parameters are shown in Table 3. Group C showed the most compromised indexes; in fact the sural potentials were not detectable in all patients of this group and the peroneal motor conduction velocity and amplitude were significantly reduced in comparison with Groups A and B.

The mean values for vibratory perception threshold are displayed in Fig. 2. The vibratory sensitivity was significantly reduced in Group C ($P < 0.05$ vs A and vs B), while Groups A and B showed similar values.

The degree of metabolic control was similar in the three groups, which showed no significant differences in fasting plasma glucose and in glycosylated haemoglobin. No significant difference in blood pressure and creatinine levels was observed in the different diabetic patients studied. Other

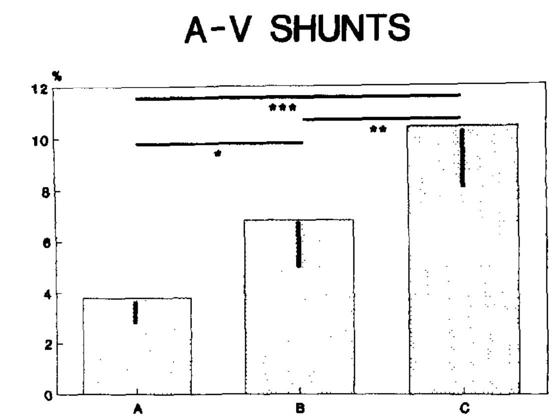

Fig. 1. Mean ± SD percentage of arterio-venous shunts in the three groups of patients (see Table 1) (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

TABLE 3

Nerve conduction values of study subjects (mean ± SD). All the nerve conduction parameters were more compromised in Group C than Groups A or B (A vs C, $^{a}P < 0.01$; $^{b}P < 0.001$. B vs C, $^{c}P < 0.05$; $^{d}P < 0.01$). In particular, the activity of the sural sensory nerve was not detectable in Group C patients. The amplitude of motor and sensory nerves was found to be reduced in Group B in comparison with Group A (A vs B, $^{e}P < 0.05$). n = 12 for each group; for characteristics, see Table 1

	Group		
	A	B	C
Number of subjects	12	12	12
Peroneal motor			
Velocity (m/s)	42 ± 3.2^{a}	40.2 ± 5.8^{d}	26.7 ± 18
Amplitude (mV)	$11.7 \pm 4.8^{b,e}$	6.9 ± 5.8^{c}	1.4 ± 0.78
Sural sensory			
Velocity (m/s)	40.4 ± 23.5	33.5 ± 23.1	Not evokable
Amplitude (μV)	12.8 ± 10.1^{e}	4.25 ± 4.8	Not evokable

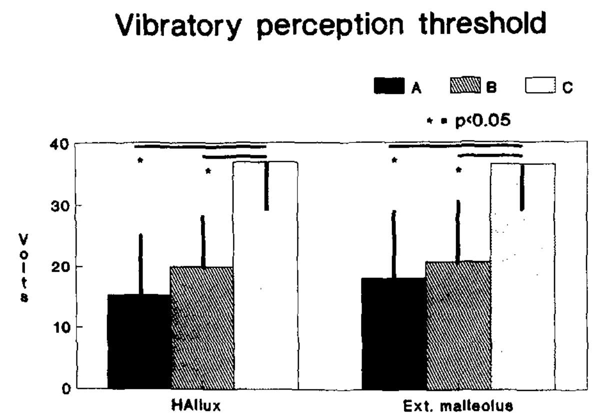

Fig. 2. Mean ± SD of the vibratory sensitivity threshold in the three groups of patients (see Table 1). The vibratory sensitivity was significantly reduced in Group C (* $P < 0.05$).

complications in diabetic patients were albuminuria (Group A, n = 4; Group B, n = 4; Group C, n = 5) and retinopathy (Group A, background n = 5, proliferative n = 3; Group B, background n = 4, proliferative n = 5; Group C, background n = 7, proliferative n = 3).

Discussion

Neuropathy can exert a pathogenetic role in the development of diabetic foot. Motor neuropathy causes atrophy of the intrinsic muscles of the foot with subsequent loss of function leading to claw or hammer toes [21]. Sensory neuropathy causes loss of pain and thermic sensibility, together with the blunting of light touch and vibration sensation [22]. Autonomic neuropathy causes lack of sweating, leading to brittle skin and altered distribution of blood flow [23]. In particular, we wish to stress the importance of autonomic neuropathy: in fact, the a-v anastomoses that allow blood to flow directly from arterioles into venous vessels are under the physiologic control of the sympathetic nervous system [24] and sympathectomy results in a complete opening of the anastomoses so that the capillary circulation is bypassed and nutritional and gaseous exchanges between blood and extravascular fluids are impaired [25].

Neuropathic foot is a condition characterized by an abnormally increased blood flow [6,26,27]. Both the bone remodelling and the structural reorganization that are characteristic of diabetic foot require an augmented blood flow to develop [28]. Reduced deoxygenation in the leg veins [5] and a typical continuous flow verified by Doppler registration have been detected in diabetic patients with neuropathic foot [6,26]. In addition, foot bones in the course of diabetic osteoarthropathy show a very high isotopic captation, thus demonstrating that the blood flow is increased in the bony tissue [29].

Until now only indirect evidence has supported the hypothesis that the impairment of the autonomic nervous system may contribute to the development of diabetic foot by the opening of shunts. Various animal experiments have shown a direct relationship between autonomic involvement in surgical sympathectomy and shunt opening. It has been shown that unilateral sympathectomy causes a 100% mean decrease in femoral vascular resistance. These changes do not occur in the non-sympathectomized limb [25]. In man, only Partsch in 1977 has shown abnormally dilated shunts in diabetic subjects [30].

We have demonstrated that the condition of diabetic foot and the presence of autonomic neuropathy are both characterized by an abnormal dilation of a-v anastomoses. We have also found a direct relationship between shunts and autonomic neuropathy; in our experience, a-v shunts showed a linear correlation with DB, which is a most sensitive, reliable and reproducible measure of the cardiovascular autonomic nervous system and provides a useful screening test to detect early autonomic dysfunction [31], and with SH and PH, tests that explore mainly the sympathetic function. This confirms the role of the sympathetic nervous system in the control of a-v anastomoses [32] and supports the hypothesis that the important alterations of peripheral circulation in diabetic foot are due to a sympathetic impairment.

In this regard it must be noted that some authors have questioned the value of cardiovascular autonomic tests in assessing diabetic autonomic neuropathy because the severity of nerve damage in the course of diabetes has been shown to vary considerably in different organs. Thus the involvement of the cardiovascular system may not necessarily reflect the involvement of other organs. However, in the absence of an accurate and universally accepted method to determine autonomic impairment in diabetes, cardiovascular autonomic tests still represent a simple and valuable tool for the study of autonomic neuropathy in diabetic patients, as indicated by a considerable number of researchers [7,33].

Data from Tooke and Tymms indicate that metabolic control may influence peripheral blood flow [34]. In our experience we could not study the influence of this variable since the three groups showed the same degree of metabolic control.

Arterio-venous shunting in Group C was greater than in Group B, while the tests exploring autonomic function were not able to differentiate between the two groups. Thus patients with a comparable degree of autonomic impairment did not show the same amount of a-v shunting. We hypothesize that local conditions present in diabetic foot (such as bone-remodelling, infections, inflammatory reactions, etc.) may explain the increased a-v shunting of Group C patients in comparison with Group B. Therefore, it would appear that while the a-v shunting induced by autonomic neuropathy may play a role in the pathogenesis of diabetic foot [35], the advanced sensory and motor neuropathy represents the real predisposing factor for the development of the overt clinical manifestations of diabetic foot. In our experience, Group C patients showed the most advanced peripheral neuropathy.

In conclusion, pathological a-v shunting seems to be a constant feature of diabetic neuropathy. The positive correlation between cardiovascular autonomic tests and the degree of a-v shunting confirms the importance of the autonomic nervous system in the regulation of peripheral blood flow and indicates that a-v shunts, which are under sympathetic control, are abnormally dilated in diabetic neuropathy. The different percentage of a-v shunts in subjects with and without foot lesions supports the hypothesis that the shunting induced by sympathetic involvement plays only a partial role in the pathogenesis of neuropathic foot lesions; and only in the presence of peripheral neuropathy may the overt clinical manifestations of diabetic foot develop.

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