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1 **The Impact of Vein Mechanical Compliance on Arteriovenous Fistula Outcomes**

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15

16 Key words: fistula; dialysis; vein compliance; outcomes

17

18

19 ***Abstract***

20

21 Purpose: Arteriovenous fistulae (AVF) are the preferred access for haemodialysis but
22 suffer a high early failure rate. The aim of this study was to determine how venous
23 distensibility, as measured in vitro, relates to early outcomes of AVF formed with the
24 sampled vein.

25

26 Methods: Ethical approval was obtained for all aspects of this study. During
27 arteriovenous fistula formation a circumferential segment of the target vein was
28 sampled. Mechanical stress testing of the venous segments was undertaken utilising a
29 dynamic mechanical analyser, with progressive stress loading at 2 N per minute to a
30 maximum of 10 N or until sample disruption. Stress strain curves were obtained for
31 vein samples and Young's modulus(YM) calculated. Duplex assessment of the fistulae
32 was undertaken at 30 days.

33

34 Results: 30 patients consented to participate with 29 samples obtained for analysis.
35 Statistical comparison of YM demonstrated no relationship with common
36 cardiovascular risk factors or dialysis status. Subject age greater than 65 was the only
37 patient factor which showed a significant difference in YM ($p=0.05$). Furthermore a
38 negative correlation was confirmed between age and YM (Pearson's $r=-0.465$,
39 [$p<0.05$]). 9 of the 29 subjects suffered an early AVF failure. Mann-Whitney U testing
40 for differences in distribution reported that YM was significantly higher in those fistulas
41 which failed ($p<0.005$).

42

43 Conclusion: Reduced venous compliance appears to result in higher failure rates of
44 arteriovenous fistulae. With the advancement of clinical tools such as speckle tracing
45 ultrasound identification of vessel compliance in vivo may produce valuable additional
46 information for clinicians planning AVF surgery.

47

48 ***Introduction***

49

50 Upper limb autologous arteriovenous Fistulae (AVF) are the preferred vascular access
51 method for chronic haemodialysis¹. Despite significant advantages over alternative methods,
52 AVF suffer particular problems such as failure to mature and early thrombosis. “Failure to
53 mature” describes insufficient flow within or dilatation of the venous segment of an AVF to
54 allow use for dialysis despite the AVF remaining patent. Failure to mature has been reported
55 in up to 10% of newly formed AVF² and as maturation relies upon increasing venous
56 diameter the distensibility of veins has previously been investigated as a potential predictor of
57 AVF success. The aim of this study was to determine how venous distensibility, as measured
58 in vitro, related to outcomes of early failure or failure to mature in AVF formed with the
59 sampled vein.

60

61 ***Materials and Methods***

62 Research Ethics Committee approval was obtained prior to study commencement and
63 informed consent was obtained from each subject. Thirty consecutive, consenting patients
64 undergoing AVF formation were assessed by physical examination and duplex ultrasound
65 (DUS) to determine the optimal site of AVF formation. At operation a distal section of vein
66 was sampled as a complete circumferential segment for testing. Samples were then snap
67 frozen to -80° centigrade for storage and transport. Freezing of samples was necessary due to
68 logistical factors and no comparisons were possible between fresh and frozen samples to
69 determine what effect storage of samples might have on results obtained.

70 The mechanical testing of vein samples was undertaken using the Dynamic Mechanical
71 Analyser (DMA) Q800 (TA Instruments, Delaware, USA). A short section of vein was
72 mounted on the test equipment using a bespoke jig manufactured specifically for this series of
73 measurements (Figure 1). The controlled force/strain rate mode of the analyser was used
74 with a preload of 0.25 Newtons (N) applied to all samples to remove slack prior to
75 commencing testing. A progressive stress loading at 2 N per minute to a maximum of 10 N or
76 until sample disruption was used.

77 Since the primary focus of the study was vein compliance in relation to early failure or failure
78 to mature follow up was limited to the 30 day post operative period. Participants were
79 assessed at 30 days (+/- 3 days) post surgery both clinically and with DUS to record the
80 diameter of the AVF draining vein as well as an estimations of AVF flow based upon peak
81 systolic velocity and end diastolic velocity. Failure to mature was defined as a fistula which
82 remained patent but had with a blood flow of $<250\text{ml}/\text{min}$ at 30 days³.

83

84 **Calculating Young's Modulus**

85 Hooke's law states that the stress applied to a material is proportional to the strain on that
86 material. In a plot of stress versus strain is therefore a region of constant slope during which
87 Hooke's law holds. The gradient of the curve at this point represents the Young's modulus
88 for the material (the ratio of stress over strain for a material). Young's modulus is the
89 recognised measure of the ease of deformation of an elastic material. A high value for
90 Young's Modulus implies an inelastic or stiff material whilst a low value suggests a very
91 flexible material.

92 Biological materials do not show the same linear stress-strain curves as engineering
93 materials. Biological materials consisting of mixed fibres will instead tend to exhibit non-
94 linear, anisotropic behaviour resulting in a stress strain curve which is typically lazy-S shaped
95 with 3 distinct regions as demonstrated in Figure 2.

96 For accurate estimation of Young's modulus, region 2 in which Hooke's law holds was
97 utilised for measurement of compliance in the tested vessels. However, estimates of Young's
98 modulus were also made in region 1 as this might allow better comparison with published
99 data for veins tested for compliance under lower loads as would be expected under tourniquet
100 testing.

101 Output data was plotted into stress strain curves using Microsoft Excel. The gradient was
102 calculated for both the initial loading in "Region 1" and also in "Region 2" of the curve
103 where a true linear relationship was seen and in which Hooke's Law holds.

104

105 **Statistical Analysis**

106 Chi-square tests were undertaken for categorical data and t-tests or Mann Whitney U tests for
107 interval data/ordinal, depending on normality of the data and type of data. All statistical
108 analyses were undertaken on SPSS 19. A p-value of <0.05 was considered to indicate
109 statistical significance.

110

111 **Results**

112 One sample proved inadequate for mechanical testing hence results for 29 samples are
113 reported. The average age of subjects was 63 years (34-83) and 23 male (79%). 21 samples

114 were from cephalic vein used in Snuff-Box or radio-cephalic fistulae in the distal arm, 7 from
115 cephalic vein used in brachio-cephalic in the antecubital fossa and 1 from a basilic vein which
116 was transposed in the subject's upper arm. Summary data for study subjects is shown in
117 Table 1.

118

119 **Compliance**

120 On analysis of the stress strain curves two samples ruptured before an obvious yield region
121 and as such resulted in a J shaped curve of region 1 and 2 only whilst the majority of samples
122 demonstrated the expected lazy S curve. Young's modulus in region 2 for the cohort of
123 samples tested returned a mean of 0.136 MPa and median of 0.122 MPa. Results had an inter
124 quartile range of 0.1680 MPa and a total range between 0.0064 MPa and 0.3564 MPa.

125 Estimation of Young's modulus in region 1 returned a mean value of 0.0164 MPa and a
126 median of 0.0079 MPa. Results had an inter quartile range of 0.0160 MPa and a total range
127 between 0.0001 MPa and 0.0777 MPa.

128 Young's Modulus in region 2 was compared between those groups of subjects with and
129 without the presence of factors which were predicted to potentially have an effect on venous
130 compliance including gender, diabetes, hypertension, smoking and dialysis status at time of
131 surgery. Those receiving forearm AVF were also compared to those receiving upper arm
132 AVF. The only factor which showed in a significant difference in Young's modulus was if a
133 subjects age was greater than 65 ($p=0.05$ – Mann Whitney U testing).

134 Further exploration of the relationship between Young's modulus and age revealed a
135 significant negative correlation between Young's modulus and age (Pearson's $r=-0.465$,
136 $p<0.05$. Figure 3)

137

138 Compliance with early AVF failure

139 9 of the 29 subjects (31%) suffered an AVF failure within 30 days, all of which were due to
140 thrombosis in the fistula vein. There were no cases of failure to mature in this series. Median
141 and interquartile range of Young's modulus measured in region 2 in samples from AVF
142 which failed was 0.2159 MPa and 0.1279 MPa compared to 0.0744 MPa and 0.1233 MPa
143 respectively in samples from AVF which remained patent. Mann-Whitney U testing for
144 differences in distribution reported a significant difference between Young's modulus in
145 region 2 in the two groups ($p < 0.005$).

146

147 Estimated Young's modulus (region 1)

148 Young's Modulus in region 1 was also compared between those groups of subjects with and
149 without the presence of factors discussed above but was not significantly different between
150 groups of subjects with or without any of these factors. Median and interquartile range of
151 Young's modulus measured in region 1 in samples from AVF which failed was 0.0157 MPa
152 and 0.0606 MPa compared to 0.007 MPa and 0.0111 MPa in samples from AVF which
153 remained patent. Mann-Whitney U testing for differences in distribution reported no
154 significant difference between Young's modulus in region 1 in the two groups ($p = 0.085$).
155 Though the low p value suggests a trend towards poorer compliance as measured in region 1
156 and AVF failure.

157

158 Discussion

159 This series has demonstrated a significant increase in directly measured vein wall stiffness in
160 AVF which suffered early failure . Previous clinical data supports this finding such as data
161 that the average tourniquet application increase in venous diameter in veins used for AVF
162 formation was only 12% in those which subsequently failed, compared to 48% in veins
163 utilised to create patent AVF ⁴. Strain-gauge plethysmography in uraemic patients has also
164 demonstrated that where venous distensibility was <0.50 mL/mmHG 100% of subsequent
165 AVF failed where as when venous distensibility was >0.50 mL/mmHg only 20% failed⁵.
166 Poor venous distensibility may also be a contributing factor in the 15 - 40% of AVF that may
167 be expected to thrombose in the early post operative period^{6 7} (i.e. primary failure) as
168 inadequate distension would result in reduced flow through the anastomosis and potentiate
169 risk of thrombosis at the site of intimal injury.

170 The underlying reasons for differences in venous compliance between patients has previously
171 been investigated in studies of arterialised venous conduits as used in coronary and lower
172 limb bypass grafting. These consistently report the presence of pre-existing disease altering
173 venous wall structure which can affect patency and contribute to the development of later
174 stenosis in both coronary and femoro-distal bypass conduits ⁸⁻¹¹. Wali et al studied the pre-
175 existing morphological changes in the wall of the cephalic vein before AVF construction and
176 compared this to vein from non uraemic upper limb vein samples ¹². Compared with normal
177 cephalic veins, all pre-access cephalic veins showed generalized intimal hyperplasia. Veins
178 from uraemic patients demonstrated loss of internal elastic lamina and endothelial cell layer,
179 mucoid or myxoid degeneration, inflammatory cell infiltration and mural calcification. This
180 suggests that macroscopically normal cephalic veins used in AVF construction are highly
181 likely to have morphological abnormalities which could adversely affect the outcome of AVF
182 surgery. When histological appearances of common and external iliac vein samples collected
183 from patients receiving renal transplantation were compared to inferior cava and common

184 iliac vein from healthy donors and cadavers, the uraemic veins demonstrated a significant
185 increase in the thickness of the venous media in hypertensive uraemic patients as compared to
186 normotensive uraemic patients and controls¹³. This would appear to suggest that the
187 cardiovascular risks factors associated with arteriosclerosis may have similar effects on the
188 venous circuit and the potential quality of vein used in access grafts.

189 The results of this study demonstrated a significant negative correlation between advancing
190 age and Young's modulus in region 2 ($p=0.05$). This appears contrary to the generally
191 accepted view of increasing vessel stiffness with increasing age. However the majority of
192 reported data relates to the arterial vessels and increasing stiffness is believed to be due to a
193 decline in the relative concentration of elastin fibres compared to collagen fibres¹⁴. This
194 decline in the elastin fibres leads to vessel ectasia and relative stiffness as the stress loading
195 of the arterial wall is taken up by inelastic collagen fibres rather than deformable elastin
196 fibres. In effect the stress-strain plot commences within region 2 rather than region 1 of the
197 lazy s curve. Since collagen is far stiffer than elastin this results in less compliant arterial
198 vessel walls with advancing age. The authors are aware of only one other series reporting
199 venous compliance in relation to age which showed an inverse relationship between age and
200 vein compliance during in vivo testing in healthy volunteers¹⁵. The explanation for this
201 difference on outcomes is unclear however the simplistic explanation of the degradation of
202 elastin and increased relative concentration to collagen may not be a complete reflection of
203 the structural changes occurring in vessel walls. Catell et al. documented a significant loss of
204 both vessel wall dry weight and mass of collagen and elastin with age. However, the
205 concentration of these fibres in the vessel wall was actually increased with increasing age.
206 This finding would suggest that there was a significant loss of other vessel wall components
207 at a rate which exceeds that at which collagen and elastin are lost.¹⁶. Furthermore, Fonseca et

208 al. studying vein samples reported that increases in age resulted in decreases in larger
209 collagen fibres, whilst no significant changes in small collagen fibres were found¹⁷.

210 This study is limited in that it was performed on in vitro specimens that had been harvested
211 and frozen prior to testing. As such the testing performed was a measurement of underlying
212 structural compliance of the vein tissue and may thus be quite different to the physiological
213 compliance of the vein in situ in vivo. It may therefore be the case that this study has shown
214 results of compliance changes with age that actually reflects the reduced supporting tissues in
215 the vessel wall or altered collagen makeup of the vessel wall. It may be that in vivo studies
216 may show increased stiffness with increasing age by measuring the mechanical effects of
217 supporting connective tissues or basal smooth muscle tone rather than simply the vessel wall
218 structural compliance as in this study.

219 Of note the rate of failure in female subjects and in those receiving an ACE inhibitor was
220 double that in males or those not prescribed ACE inhibition. Females are known to suffer
221 failure more commonly than males¹⁸ and prescription of ACE inhibition may reflect a higher
222 cardiovascular risk profile. This small study is not powered to explore this finding in any
223 detail.

224 The clinical relevance of the results reported here is that any bedside or non invasive test of
225 venous compliance which could be performed prior to AVF formation may have significant
226 bearing on the site chosen or the management of the subsequent AVF. Tests available are
227 limited at present in most units but simple bedside measurement of percentage change in vein
228 diameter with and without tourniquet application has shown promise in predicting AVF
229 Patency⁴. However, distension under tourniquet likely represents stresses as might be
230 expected in region 1 of the stress strain curve with relatively low venous pressures. As a vein
231 is utilised in an AVF, higher stress loading of the vessel wall would be expected due to

232 arterial pressures applying radial loading to the vessel. More accurate estimations of vein
233 compliance could be achieved using using strain-gauge plethysmography⁵ but this technique
234 is complex to perform and remains in the realm of research laboratories at present.

235 Elastography and ultrasound speckle tracing is a recently developed technique utilising high
236 frequency ultrasound to apply mechanical compression or vibration to tissue and then detect
237 the extent of resultant deformation. By utilising simplifying assumptions, the deformation can
238 then be interpreted as representative of the underlying Young's modulus or compliance of the
239 tissue. Early studies utilising these techniques in assessing vein compliance in the forearm
240 suggest it may provide detailed, high-resolution and spatially accurate maps of vein-wall
241 mechanics¹⁹. Assessment of stenoses in AVF draining veins with elastography reported
242 lower values of strain in stenotic regions compared to the remainder of the vessel, indicating
243 greater stiffness of the vessel wall at these points²⁰. The non invasive ultrasound speckle
244 tracking techniques described in these papers has clear potential to determine vein wall
245 mechanical properties noninvasively, and may in the future become an adjunct to AVF
246 planning.

247 ***Conclusion***

248 The histological basis for changes in compliance both with age and between subjects
249 warrants further investigation. With the advancement of clinical tools such as speckle tracing
250 ultrasound identification of vessel compliance in vivo may produce valuable additional
251 information for clinicians planning AVF surgery.

252

253

254

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312

313

Variable	Failures with variable present	Failures with variable absent	Young's Modulus with variable present			Young's Modulus with variable absent			Difference
			n=	Median	IQR	n=	Median	IQR	Signif (p=)
Age >65	2/ 15 (13%)	7/ 14 (50%)	15	0.0907	<i>0.1304</i>	14	0.1756	<i>0.1458</i>	>0.05
Male gender	6/23 (26 %)	3/6 (50 %)	23	0.0734	<i>0.2369</i>	6	0.1455	<i>0.1597</i>	0.896
Diabetes	2/10 (20%)	7/19 (36%)	10	0.1391	<i>0.1168</i>	19	0.0918	<i>0.1927</i>	0.429
Hypertension	3/ 13 (23%)	6/ 16 (37%)	13	0.0918	<i>0.1674</i>	16	0.1467	<i>0.1885</i>	0.449
Smoking	4/ 12 (33%)	5/ 17 (29%)	12	0.1339	<i>0.1190</i>	17	0.1131	<i>0.1941</i>	0.879
ACEi	2/ 4 (50%)	7/ 25 (28%)	4	0.0909	<i>0.1572</i>	25	0.1455	<i>0.1752</i>	0.444
Statin	3/ 11 (27 %)	6/ 18 (33%)	11	0.091	<i>0.1905</i>	18	0.1467	<i>0.1655</i>	0.492
On Dialysis	5/ 16 (31%)	4/ 13 (30%)	16	0.1024	<i>0.1191</i>	13	0.1904	<i>0.1737</i>	0.374

Table 1 -Comparison of Young's modulus with and without various factors (n- number, SD, standard deviation, ACEi – Angiotensin converting enzyme inhibitors)

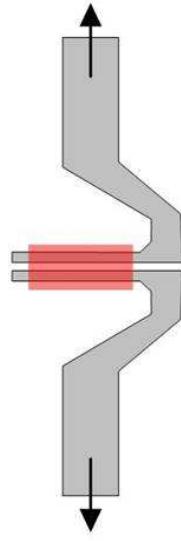


Figure 1 - Jig for mechanical deformation testing of vein samples – the red section shows the testing position of the specimen of vein with the pins of the clamps both passing through the vein lumen.

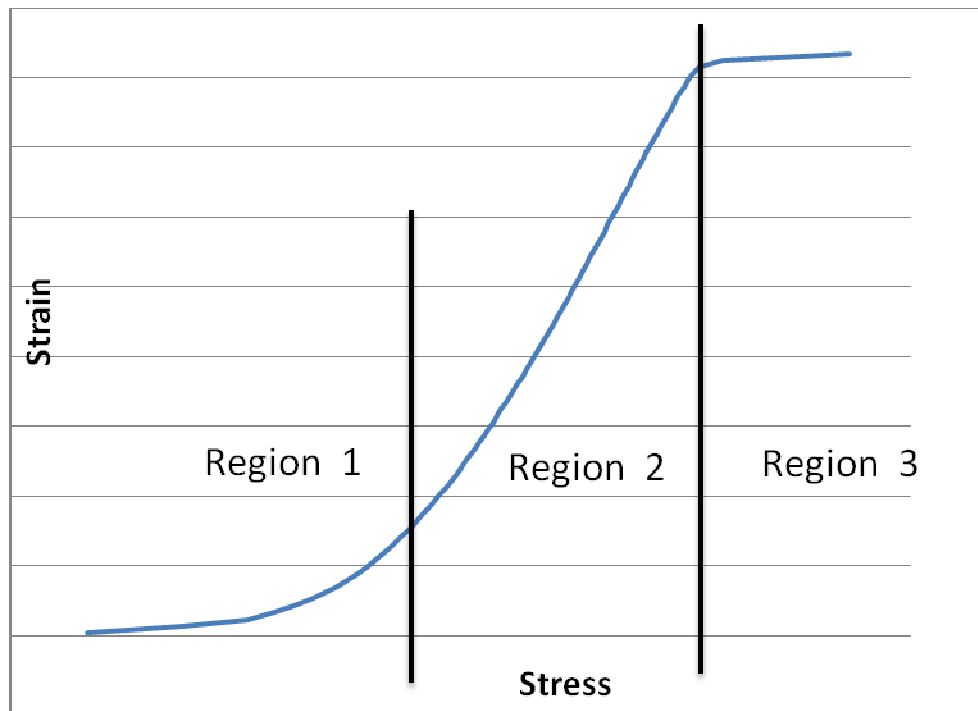


Figure 2 – Typical stress-strain curve for soft tissues under increasing loads showing 3 distinct regions of response

Region 1 - Mainly accounts for stretch of the interlaced elastin fibres, and in this region the collagen fibres are not yet aligned with the load and remain largely unaffected¹⁹.

Region 2 – As further loading occurs, elastin fibres are fully aligned and collagen fibres are now in the direction of the force and begin to bear load. In this region the tissue exhibits a near linear stress-strain relationship and this represents the mechanical elastic modulus or young's modulus of the tissue¹⁹.

Region 3 – At high loads the Collagen fibres reach full stretch and strongly resist further deformation causing the tissue to behave in a very stiff manner. This is the “yield” region and as load increases, the structure of the tissue begins to permanently alter as collagen fibres start to break¹⁹.

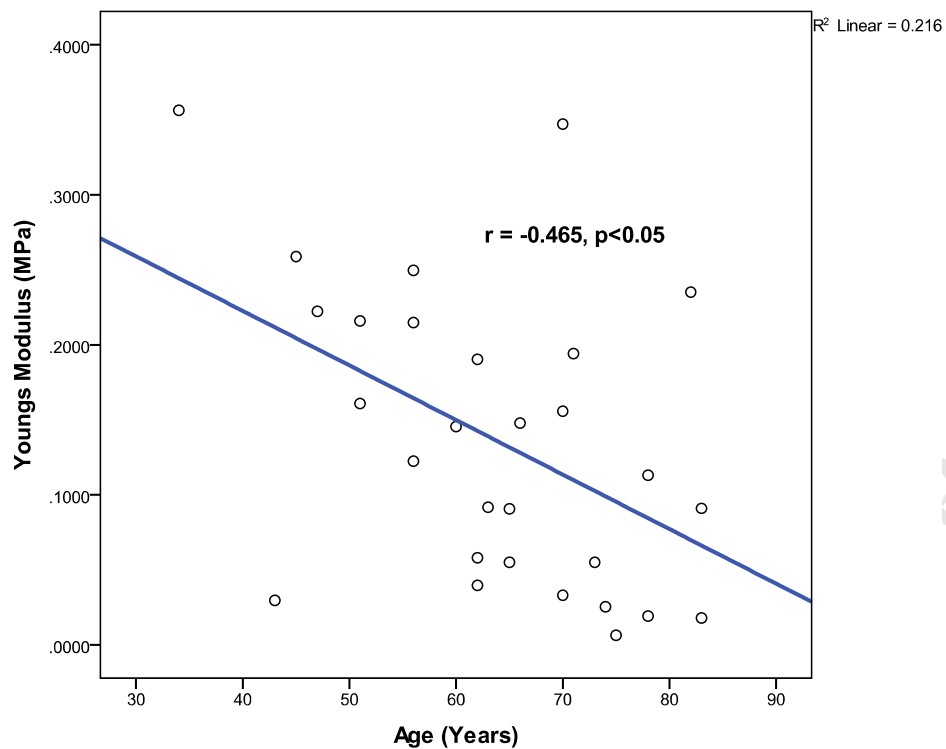


Figure 3 - Young's Modulus plotted against subjects age (line of best fit indicates significant negative correlation) (P value shown calculated using Pearson's test for correlation)