

## Theme Issue Article

# Circulating endothelial cells in acute ischaemic stroke

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### Summary

Increased numbers of CD146-bearing circulating endothelial cells (CECs) in the peripheral blood probably represent the most direct evidence of endothelial cell damage. As acute ischaemic strokes are associated with endothelial abnormalities, we hypothesised that these CECs are raised in acute stroke, and that they would correlate with the other indices of endothelial perturbation, i.e. plasma von Willebrand factor (vWf) and soluble E-selectin. We studied 29 hypertensive patients (19 male; mean age 63 years) who presented with an acute stroke and compared them with 30 high risk hypertensive patients (21 male; mean age 62 years) and 30 normotensive controls (16 male; mean age 58 years). CECs were estimated by CD146 immunobead capture, vWf and soluble E-selectin by ELISA. Patients with an acute ischaemic stroke had significantly higher

numbers of CECs/ml of blood ( $p < 0.001$ ) plasma vWf ( $p = 0.008$ ) soluble E-selectin ( $p = 0.002$ ) and higher systolic blood pressure (SBP) as compared to the other groups. The number of CECs significantly correlated with soluble E-selectin ( $r = 0.432$ ,  $p < 0.001$ ) and vWf ( $r = 0.349$ ,  $p = 0.001$ ) but not with SBP ( $r = 0.198$ ,  $p = 0.069$ ). However, in multivariate analysis, only disease group (i.e. health, hypertension or stroke) was associated with increases CECs. Acute ischaemic stroke is associated with increased numbers of CECs. The latter correlate well with established plasma markers of endothelial dysfunction or damage, thus unequivocally confirming severe vasculopathy in this condition. However, the greatest influence on CECs numbers was clinical group.

### Keywords

Endothelial damage, acute ischaemic stroke, hypertension, circulating endothelial cells, CD146

Thromb Haemost 2005; 94: ■

### Introduction

As the majority of strokes are atherothrombotic in origin, the final precipitating event being the formation of an occlusive thrombus in the intra-cerebral arteries, it would therefore be expected that patients with stroke (or the risk factors predisposing to stroke, such as hypertension) would demonstrate abnormalities of haemostasis, rheology and vascular function that predispose to such a prothrombotic state (1, 2). Indeed, abnormalities of coagulation factors, platelets and endothelial markers are present in patients who have suffered a stroke, although whether or not this is cause or effect is unclear (3–5). However, a likely reason for hypertension being the primary risk factor for stroke is that the former is itself associated with abnormalities in the endothelium (such as increased von Willebrand factor and soluble E selectin, and impaired flow mediated dilatation) along with changes in coagulation factors (e.g. raised fibrinogen) and platelets (e.g. increased soluble P selectin) that together bring a prothrombotic state, suggesting that these abnormalities are (at least in part) causative and/or contributory to stroke (1, 6, 7).

The evaluation of the number of circulating endothelial cells (CECs) in peripheral blood is a relatively new method for assessing endothelial damage. It is presumed that these once mural cells, identified by the presence (for example) of von Willebrand factor, are driven (i.e. are desquamated) from the blood vessel wall by the particular disease process and are thus detected in the plasma (8). The greatest abnormalities in CEC counts are seen in severe and/or acute conditions such as acute coronary syndromes and myocardial infarction, inflammatory vasculitis and bone marrow transplantation (9–11). In peripheral artery disease, where the highest levels are found in critical limb ischaemia, CECs correlate with von Willebrand factor (12). Increased numbers of CECs have also been described in pulmonary hypertension, where they correlate with systolic, diastolic and mean pulmonary artery pressures (13). Approximately 25% of these CECs expressed E-selectin, implying that they have been immunologically activated.

As a thrombotic stroke is the most severe manifestation of target organ damage in hypertension, we hypothesised that CECs are raised in patients with an acute stroke, and that they would

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Received December 13, 2005  
Accepted after resubmission May 15, 2005

Prepublished online ■, 2005 DOI: 10.1160/TH04-12-0795

correlate with established plasma indices of endothelial perturbation, that is, plasma von Willebrand factor and soluble E-selectin levels. To test this hypothesis, we performed a cross-sectional study of patients who had recently suffered a stroke, and compared them to patients with established systemic hypertension but no evidence of major cardiovascular/cerebrovascular disease, and with healthy normotensive controls.

## Materials and methods

We investigated patients within 24 hours after an acute ischemic stroke, and compared them with healthy age-, sex- and ethnic-matched control subjects, and hypertensive patients without evidence of cardiovascular or cerebrovascular disease as 'risk factor' control subjects. Stroke diagnosis was based on the WHO criteria: rapidly developing clinical signs of focal and, at times, global loss of cerebral function with symptoms lasting more than 24 h or leading to death, with no apparent cause other than that of vascular origin. Patients with a previous stroke were eligible for inclusion if the previous event was remote (>3 months) and the new, acute presentation involved a new neurological 'territory', based on clinical assessment. The precise onset of ictus was ascertained by careful clinical history from the patient, and possibly relatives, as well as review of the general practitioner referral letter. All patients underwent computed tomography scanning (CT scan) to define the type of stroke and all received aspirin for secondary prevention, but no heparin, as per hospital policy. Table 1 summarises the demographic and clinical data of the study groups. The study was approved by the West Birmingham research ethics committee. All subjects gave informed consent,

and were stroke patient was not able to give consent, assent was obtained from the next of kin.

Subjects with essential hypertension (defined as >160/95mmHg, on hospital or general practitioner records, or taking antihypertensive treatment) were recruited from our Out-Patient clinics. Subjects with a past history of ischaemic heart disease (IHD), stroke or peripheral vascular disease (PVD) were excluded from this group. In both patient groups, vascular risk factors were noted: arterial hypertension (defined as above); diabetes mellitus (taking anti-diabetic treatment, elevated haemoglobin A<sub>1c</sub>, or elevated blood glucose at 2 readings prior to admission with stroke), and hyperlipidaemia (on lipid-lowering medication or total cholesterol >5.7 mM/220 mg/dl or triglycerides >4.7 mM/180 mg/dl before stroke). The healthy subjects were recruited from hospital staff, relatives of the patients or those attending the hospital for routine cataract or hernia surgery. They were not taking any regular medication, were normotensive (<140/85 mm Hg) and in sinus rhythm.

For all the categories of subjects, exclusion criteria were trauma, surgery, or acute organ ischaemia (e.g. myocardial infarction, unstable angina (9), critical limb ischaemia (12)(all possibly leading to raised CECs)) within the preceding 3 months, severe liver disease, renal failure, malignancies; chronic inflammatory diseases (e.g. rheumatoid arthritis); and fever or acute inflammatory or infectious conditions (e.g. requiring antibiotics) at the time of entry to the study. Despite this we acknowledge the likelihood that patients may well have non-symptomatic atherosclerosis of various vascular beds, including the coronary circulation.

**Table 1: Details of the study groups.** All values are number (percentage) or mean (standard deviation). Analysis by Chi-square test, or one-way ANOVA as appropriate.

|                                | Normal controls<br>(n=30) | Hypertensive controls<br>(n=30) | Acute strokes<br>(n=29) | p-value |
|--------------------------------|---------------------------|---------------------------------|-------------------------|---------|
| <b>Demography</b>              |                           |                                 |                         |         |
| Age (years)                    | 58 [6]                    | 62 [8]                          | 63 [9]                  | 0.051   |
| Sex (Male:female)              | 16:14                     | 21:9                            | 19:10                   | 0.08    |
| <b>Drug therapy</b>            |                           |                                 |                         |         |
| CCB                            | (none)                    | 10 (33%)                        | 8 (27%)                 | 0.7     |
| Beta blockers                  |                           | 13 (43%)                        | 5 (17%)                 | 0.07    |
| ACEI                           |                           | 8 (27%)                         | 9 (30%)                 | 0.5     |
| Diuretics                      |                           | 16 (52%)                        | 9 (30%)                 | 0.1     |
| Aspirin                        |                           | 6 (20%)                         | 10 (33%)                | 0.2     |
| Statin                         |                           | 11 (37%)                        | 7 (23%)                 | 0.5     |
| <b>Clinical and laboratory</b> |                           |                                 |                         |         |
| Previous IHD                   | 0                         | 0                               | 7 (23%)                 | 0.01    |
| Previous CVA                   | 0                         | 0                               | 4 (13%)                 | 0.1     |
| Previous PVD                   | 0                         | 0                               | 1 (3%)                  | 0.8     |
| SBP (mmHg)                     | 131 [15]                  | 143 [17]                        | 154 [29]                | 0.009   |
| DBP (mmHg)                     | 80 [9]                    | 80 [10]                         | 80 [13]                 | 0.74    |
| Smokers                        | 2 (8%)                    | 5 (16%)                         | 2 (7%)                  | 0.28    |
| Diabetics                      | 0                         | 5 (16%)                         | 3 (10%)                 | 0.48    |
| Glucose (mM)                   | 5.0 [0.7]                 | 6.1 [1.8]                       | 6.2 [1.3]               | 0.002   |
| Cholesterol (mM)               | 5.2 [0.9]                 | 5.5 [1.0]                       | 5.4 [1.0]               | 0.21    |
| Creatinine (mM)                | 78 [18]                   | 103 [11]                        | 90 [33]                 | 0.001   |

## Laboratory

In the stroke patients, blood samples were taken within 24 hours of ictus. Venipuncture of forearm veins was performed with minimal stasis and blood was collected into citrated vacutainers for measurement of soluble E-selectin and von Willebrand factor and into vacutainers containing EDTA for the estimation of CECs. Blood pressure for each participant was recorded immediately prior to venipuncture. Citrated vacutainers were immediately placed on ice and within one hour were centrifuged at 3000 rpm (1000g) for 15 minutes. The plasma so obtained was then stored at  $-70^{\circ}\text{C}$  for batch analysis. Plasma levels of soluble E-selectin and von Willebrand factor were estimated by ELISA (R&D Systems, Abingdon, UK; Dako, Ely, UK). Intra-assay coefficients of variation (CV) were  $<5\%$ , inter-assay CVs were  $<10\%$  ( $n=20$  determinations each).

Estimation of CECs has been described fully elsewhere (9–13,18–20). In brief, 4 ml of blood collected in EDTA tubes was mixed with 4ml of normal saline. To this mixture, 100  $\mu\text{l}$  of a suspension of monodispersed magnetic 4.5  $\mu\text{m}$  diameter polystyrene beads (Dynabeads M-450, Dynal A.S., Oslo, Norway) coated with a secondary layer of S-Endo 1 (a monoclonal antibody recognising endothelial specific CD146 [Biocytex, Marseille, France]) was added. This mixture was incubated at room temperature for 30 minutes whilst being gently rotated (30 rpm) to ensure continued mixing. The rosetted beads were separated from the blood using an MPC-L concentrator (Dynal) and washed a total of four times. The resulting rosetted cells and beads were finally re-suspended in  $\sim 30 \mu\text{l}$  of PBS for counting by a single observer under epifluorescence microscopy (Zeiss, Welwyn Garden City, UK). CECs are easily located as they are autofluorescent although there is considerable fluorescent debris. The latter is easily identified by non-cellular morphology and intense fluorescence. The criteria for confirmation of a CEC was binding  $\geq 4$  beads and a clear and regular cell morphology of greater than 20  $\mu\text{m}$  diameter (approximately four times bead diameters), or 10 beads with an irregular morphology. For aggregated cells, the aggregate was counted as a single cell. Intra- ( $n=40$ ) and inter-assay ( $n=20$ ) coefficients of variation were  $<5\%$  and  $<10\%$  respectively. The inter- and intra-observer variations of the method in our laboratory were  $<5\%$  ( $n=20$  determinations). All laboratory work was performed in a blinded fashion with respect to the identity of the samples.

## Power calculation and statistics

Previous work has described raised CECs in cardiovascular disease that exceed those of healthy controls by a factor of 3.5–7.5 (9, 12). Bull et al (13) found levels to be increased almost ten fold in pulmonary hypertension. We therefore conservatively hypothesised that levels would be raised by a factor of two in hypertension compared to healthy controls (i.e.  $p<0.05$ ), additionally and a factor of four in stroke compared to healthy controls ( $p<0.01$ ,  $p<0.05$  to hypertension). As the distribution of CECs is non-normal (9–13) we modelled a median of 2 cells/ml (inter-quartile range 1.2–7.2) in healthy controls, rising to 4 cells/ml (2.4–14.4) in hypertension and again to 8 cells/ml (4.8–28.8) in stroke. To achieve a minimum of  $p<0.05$  between the three groups (ANOVA  $p<0.001$  overall), our model required  $n=28/\text{group}$ . We therefore recruited consecutive patients until

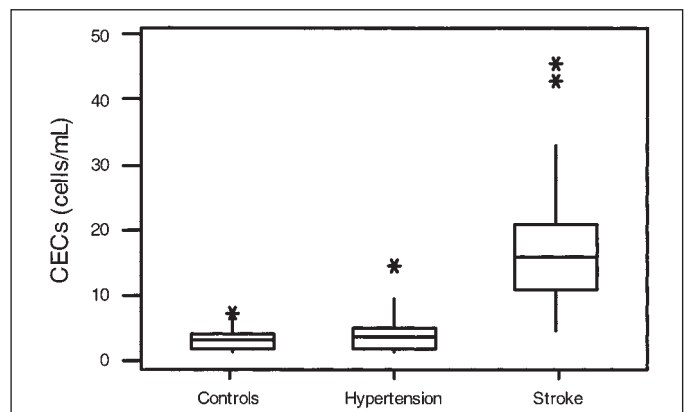
just over this number.

Categorical data were compared using the  $\chi^2$  test. Continuous data were subjected to the Ryan-Joiner test to assess distribution. Data are presented as mean and standard deviation, or median and inter-quartile range, as appropriate. The Kruskal-Wallis test was used to compare different groups where the data were not normally distributed and the one-way ANOVA for normally distributed data. Post-hoc analysis was done using the Tukeys test when the one way ANOVA was used. Correlations were done using Pearsons coefficient of correlation for normally distributed data, while Spearmans coefficient of correlation was used for non-normally distributed data. A multivariate regression model sought relationships between (log) CECs, von Willebrand factor, soluble E selectin, systolic blood pressure and clinical group. All analyses and the power calculation model were performed on Minitab release 13 (Minitab Inc, State College, PA, USA).

## Results

Patients in all three groups were of similar age and sex (Table 1). As expected, mean systolic blood pressure levels were highest in the acute stroke and hypertension groups. Glucose was higher in the patient groups, probably because of the few diabetics. Renal function was equally impaired in the patient groups compared to the healthy controls. Patients with “uncomplicated hypertension” did not have previous vascular disease (stroke, ischaemic heart disease or peripheral vascular disease). There were, however, a few patients in the acute stroke group who had previous ischaemic heart disease, previous stroke and peripheral vascular disease. There were no patients with congestive cardiac failure or renal failure. The drug therapy is given in Table 1. There were no differences in any of the research indices according to different therapies.

Patients with an acute stroke had the highest levels of plasma von Willebrand factor, soluble E-selectin and CECs (Table 2), when compared to the other two patient groups. CECs in acute stroke exceed those in the other two groups by a factor of 5, a difference well within our power requirement (Fig. 1). CECs in the



**Figure 1: Box and whisker plot showing circulating endothelial cells in patients with acute stroke, hypertension and healthy controls.** Box plot shows median and inter-quartile range, whiskers are full range, \*are distant outliers.

**Table 2: Plasma von Willebrand factor, soluble E-selectin and circulating endothelial cells in patients with acute stroke, hypertension and healthy controls.** All values are mean (standard deviation) or Median (Interquartile range). Analysis by one-way ANOVA or the Kruskal-Wallis test as appropriate. <sup>(a-c)</sup>Tukeys post hoc analysis between groups. <sup>(a)</sup> p=0.03 between controls and hypertensives, and p<0.001 between controls and strokes, and p=0.01 between hypertensives and strokes. <sup>(b)</sup> p<0.001 between all three groups, <sup>(c)</sup> p<0.001 between strokes and hypertensives and strokes and controls, p=0.9 between controls and hypertensives.

|                            | Normal controls (n=30) | Hypertensive controls (n=30) | Acute strokes (n=29) | p-value               |
|----------------------------|------------------------|------------------------------|----------------------|-----------------------|
| vWf (IU/ml)                | 117 [17]               | 133 [21]                     | 150 [29]             | <0.001 <sup>(a)</sup> |
| Soluble E-selectin (ng/ml) | 33 [14]                | 66 [31]                      | 91 [17]              | <0.001 <sup>(b)</sup> |
| CECs (/ml)                 | 2.7 (1.6-3.7)          | 3.1 (1.6-4.8)                | 15.5 (10.8-20.7)     | <0.001 <sup>(c)</sup> |

hypertensive group were not statistically different to controls. Both von Willebrand factor and soluble E-selectin levels were higher in the acute stroke patients, when compared to hypertensive group. Both plasma indices were higher in the hypertensive group compared to controls.

Of the 29 patients suffering an acute stroke, 10 had evidence of a previous cardiovascular event, and 19 were free of any event (Table 1). There were no statistically significant differences in blood pressure or the research indices between these two groups, i.e. mean (SD) SBP 156 mm Hg (35) with a history versus 153 (27) with no history (p=0.8), DBP 78 mm Hg (8) versus 82 (15)(p=0.4), von Willebrand factor 138 (31) IU/dl versus 156 (27)(p=0.1), soluble E-selectin 91 (18) versus 91 (20)(p=0.9), and CECs median (IQR) 15 cells/ml (10–21) versus 17 (12–21)(p=0.7).

In the entire subject cohort, there were significant Spearman rank correlations between CECs and the levels of von Willebrand factor and soluble E-selectin, but not with systolic blood pressure (Table 3). The correlation coefficient (rho) between diastolic blood pressure and CECs was 0.008 (p=0.943). Other correlations (age, sex glucose, creatinine) were not significant (data not shown). To determine factors that may predict or be (partially) causative of raised (log) CECs, those significant (p<0.05) in univariate analysis (i.e. systolic blood pressure, von Willebrand factor, soluble E selectin and clinical group) were entered into a multivariate regression model. The only independent predictor of CECs was clinical group (p<0.001, with a variance (R<sup>2</sup>) of 48.6%). After removal of clinical group, only soluble E selectin was significantly (p=0.002, R<sup>2</sup>=25.3%) related to CECs.

## Discussion

Endothelial damage/dysfunction in cardiovascular disease is being increasingly recognised as a contributor to major end

**Table 3: Correlations in the total cohort of patients.** Correlation of variables calculated by Spearman's ranked correlation coefficient.

|                       | Von Willebrand factor | Soluble E-selectin | CECs               |
|-----------------------|-----------------------|--------------------|--------------------|
| Systolic BP           | R=0.232<br>P=0.033    | R=0.258<br>P=0.017 | R=0.198<br>P=0.069 |
| Von Willebrand factor |                       | R=0.488<br>P<0.001 | R=0.349<br>P=0.001 |
| Soluble E-selectin    |                       |                    | R=0.432<br>P<0.001 |

points such as stroke and myocardial infarction, possibly because such dysfunction promotes thrombosis and hypertension (7, 14, 15). Assessment has focussed on plasma markers von Willebrand factor and soluble E selectin, physiological changes such as impaired flow mediated dilatation (16, 17), and the recent development of CECs (8–13). Our present CECs data adds to that in coronary artery disease (9, 18, 19), peripheral artery disease (12) and congestive heart failure (20) with raised numbers in acute stroke.

Raised levels of CECs correlate with plasma endothelial markers von Willebrand factor (as in peripheral artery disease (12) and congestive heart failure (20)) and (more strongly) with soluble E selectin. The former is an established predictor of adverse cardiovascular events (21) and increased numbers of CECs also carry a poor prognosis (18, 19). Both raised von Willebrand factor and soluble E selectin (5, 17, 22–25) are widely reported raised in stroke and in hypertension and our data confirm this. However, lack of raised CECs in hypertension is notable (Table 2, Fig. 1). Firstly it suggests to us that the damage to the endothelium in hypertension, although evidenced by raised plasma markers, is not so severe that it desquamates mural endothelial cells. Secondly, it follows that if this is the case then the profound pathological events of the acute stroke itself have caused the appearance of the CECs in the blood. However, we concede that raised CECs may precede the stroke or reflect underlying pathology such as a subtle sub-clinical infection or concurrent cardiovascular disease, the latter likely to be most prevalent in this population (26, 27). Indeed, soluble E selectin itself may reflect immunological or inflammatory activation of the endothelium (28) although the further significance of this is unclear.

The precise origin of these CECs (as in other conditions) is obscure, and we do not presume we are witnessing the destruction of the cerebral endothelium. In acute coronary syndromes, CECs are almost entirely CD34 (endothelial progenitor cell, EPC), CD36 (microvascular cells) and CD45 (leukocyte common antigen) negative but are endothelial nitric oxide synthase and von Willebrand factor positive (18, 19). Thus, as we have not immunophenotyped CECs in the current study, we cannot assume that (some of) our cells are not part of a repair process and are, in fact, part of the EPCs family of cells. Indeed, there is evidence, albeit from a highly-selected cell group, that a proportion of CD34 +ve cells also express CD146 (29). Furthermore, CD34 has in several studies been used as a marker of microvessels (30–32). Lack of data of CD146 expression on cerebral macro- or micro-vessels provides additional uncertainty. We therefore

emphasise that we cannot be sure of the origins of these cells although, as atherosclerosis is a global disease, we suspect these CECs arise from numerous anatomical sites. Although CD146 has been noted on smooth muscle cells and follicular dendritic cells, the endothelial nature of CECs is unequivocal (8–13, 18–20, 33).

Our data may, at first sight, seem to be at variance with that of Bull et al (13) who reported raised CECs in pulmonary hypertension. However, this type of hypertension is likely to be more severe towards a defined organ (i.e. the lung) whereas our systemic hypertension probably influences many vessels and vascular beds. The same argument can, of course, be applied to the source of raised plasma von Willebrand factor and raised soluble E selectin. Furthermore, many of the CECs described by Bull et al (13) stained positive for membrane E-selectin, although we can only speculate that the raised soluble E selectin arises from CECs and/or from mural endothelial cells. A further explanation is that the aetiopathology of pulmonary hypertension is diverse and partly of microvascular thrombotic origin.

The clinical consequences of increased numbers of CECs in the plasma are slowly becoming clear (34,35). Two groups have recently shown that high levels are associated with poor outcome in acute coronary syndromes (18, 19). It seems unlikely that, given the considerable mass of mural endothelium, the small numbers in the blood we observe have any direct pathophysiological repercussion for blood pressure control. However, increased CECs may well be a further generalised index of (the loss of) vascular integrity and functioning, as are von Willebrand factor and soluble E selectin. Nevertheless, CECs may retain

some pro-thrombotic activity, as they may express tissue factor (9, 33, 36). However, although increased von Willebrand factor is more likely to be directly implicated in adverse thrombotic events as it not only reflects vascular damage but retains the ability to promote thrombosis (37).

Our study is limited by its cross sectional design, although it is well powered to detect the difference reported with confidence. Nevertheless we were unable to obtain serial samples to support the feasible hypothesis that successful recovery would be associated with a reduction in the number of CECs. In conclusion, we have demonstrated raised CECs in patients presenting with an acute ischaemic stroke, but not in hypertensives who are free of target organ damage. We suggest that this may have significant implications in the management and prognosis of patients who present with acute stroke.

### Acknowledgements

We acknowledge the support of the City Hospital Research and Development Programme and the Haemostasis Thrombosis and Vascular Biology Unit.

### Abbreviations

SBP- Systolic blood pressure; DBP- Diastolic blood pressure; CCB- Calcium channel blockers; ACEI- Angiotensin converting enzyme inhibitor; CVA- cerebrovascular accident, IHD- Ischaemic heart disease; PVD- Peripheral vascular disease; n/a = not applicable; CECs: Circulating endothelial cells; vWf: Von Willebrand factor; R = correlation coefficient. P= probability

### References

- Lee AJ. The role of rheology and haemostatic factors in hypertension. *J Human Hypertens* 1997; 11: 767–76.
- Lane DA, Wolff S, Ireland H et al. Activation of coagulation and fibrinolytic systems following stroke. *Br J Haematol* 1983; 53: 655–8.
- Lip GYH, Blann AD, Farooqi IS et al. Abnormal haemorrhage, endothelial function and thrombogenesis in relation to hypertension in acute (ictus <12 hours) stroke patients. *Blood Coagul Fibrinolysis* 2001; 12: 307–15.
- Bath PMW, Blann A, Smith N et al. Von Willebrand factor, P-selectin and fibrinogen levels in patients with acute ischaemic and haemorrhagic stroke, and their relationship with stroke subtype and functional outcome. *Platelets* 1998; 9: 155–9.
- Liu L, Lin Z, Shen Z. Changes in von Willebrand factor and antithrombin III levels in acute stroke: difference between thrombotic and haemorrhagic stroke. *Thromb Res* 1993; 72: 353–8.
- Felmeden DC, Spencer CG, Chung NA et al. Relation of thrombogenesis in systemic hypertension to angiogenesis and endothelial damage/dysfunction (a substudy of the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT)). *Am J Cardiol* 2003; 92:400–5.
- Widlansky ME, Gokce N, Kearney JF, Jr. et al. The clinical implications of endothelial dysfunction. *J Am Coll Cardiol* 2003; 42: 1149–60.
- Woywodt A, Bahlmann FH, De Groot K et al. Circulating endothelial cells: life, death, detachment and repair of the endothelial cell layer. *Nephrol Dial Transplant* 2002; 17: 1728–30.
- Mutin M, Canavy I, Blann A et al. Direct evidence of endothelial injury in acute myocardial infarction and unstable angina by demonstration of circulating endothelial cells. *Blood* 1999; 93: 2951–8.
- Woywodt A, Streiber F, De Groot K et al. Circulating endothelial cells as markers for ANCA-associated small-vessel vasculitis. *Lancet* 2003; 361: 206–10.
- Woywodt A, Scheer J, Hambach L et al. Circulating endothelial cells as a marker of endothelial damage in allogeneic hematopoietic stem-cell transplantation. *Blood* 2004; 103: 3603–5.
- Makin AJ, Blann AD, Chung NA et al. Assessment of endothelial damage in atherosclerotic vascular disease by quantification of circulating endothelial cells; Relationship with von Willebrand factor and tissue factor. *Eur Heart J* 2004; 25: 371–6.
- Bull TM, Golpon H, Hebbel RP et al. Circulating endothelial cells in pulmonary hypertension. *Thromb Haemost* 2003; 90: 698–703.
- Folkow B. Hypertension and endothelial dysfunction – aspects of atheroma protection. *Blood Pressure* 1992; suppl 1: 11–12.
- Drexler H. Endothelial dysfunction: clinical implications. *Prog Cardiovasc Dis* 1997; 4: 287–324.
- Blann AD, Lip GY. The endothelium in atherothrombotic disease: assessment of function, mechanisms and clinical implications. *Blood Coagul Fibrinolysis* 1998; 9: 297–306.
- Felmeden DC, Blann AD, Spencer CG et al. A comparison of flow-mediated dilatation and von Willebrand factor as markers of endothelial cell function in health and in hypertension: relationship to cardiovascular risk and effects of treatment. *Blood Coagul Fibrinolysis* 2003; 14:425–31.
- Lee KW, Lip GY, Tayebjee M et al. Circulating endothelial cells, Von Willebrand Factor, interleukin-6 and prognosis in patients with acute coronary syndromes. *Blood* 2005;105:526–32.
- Quilici J, Banzet N, Ambrosi P et al. Circulating endothelial cell count as a diagnostic marker for non-ST elevation acute coronary syndromes. *Circulation* 2004; 110: 1586–91.
- Chong AY, Blann AD, Patel J et al. Endothelial dysfunction and damage in congestive heart failure. relation of flow-mediated dilatation to circulating endothelial cells, plasma indexes of endothelial damage, and brain natriuretic peptide. *Circulation* 2004; 110: 1794–8.
- Thompson SG, Kienast J, Pyke SDM, Haverkate F et al. Haemostatic factors and the risk of myocardial infarction or sudden death in patients with angina pectoris. *New Engl J Med* 1995; 332: 635–41.
- Roldan V, Marin F, Lip GY et al. Soluble E-selectin in cardiovascular disease and its risk factors. A review of the literature. *Thromb Haemost* 2003; 90: 1007–20.
- Preston Preston RA, Ledford M, Materson BJ et al. Effects of severe, uncontrolled hypertension on endothelial activation: soluble vascular cell adhesion molecule-1, soluble intercellular adhesion molecule-1 and von Willebrand factor. *J Hypertens* 2002; 20: 871–7.
- Bitsch A, Klene W, Murtada L et al. A longitudinal prospective study of soluble adhesion molecules in acute stroke. *Stroke* 1998; 29: 2129–35.
- Fassbender K, Mossner R, Motsch L et al. Circulating selectin- and immunoglobulin-type adhesion molecules in acute ischemic stroke. *Stroke* 1995; 26: 1361–4.
- Pop GA, Koudstaal PJ, Meeder HJ et al. Predictive value of clinical history and electrocardiogram in pa-

tients with transient ischemic attack or minor ischemic stroke for subsequent cardiac and cerebral ischemic events. The Dutch TIA Trial Study Group. *Arch Neurol* 1994; 51: 333–41.

27. Gates P, Peppard R, Kempster P et al. Clinically unsuspected cardiac disease in patients with cerebral ischaemia. *Clin Exp Neurol* 1987; 23: 75–80.

28. Pigott R, Dillon LP, Hemingway IH et al. Soluble forms of E-selectin, ICAM1 and VCAM1 are present in the supernatants of cytokine activated cultured endothelial cells. *Biochem Biophys Res Comm* 1992; 187: 5849.

29. Burger PE, Coetzee S, McKeehan WL et al. Fibroblast growth factor receptor-1 is expressed by endothelial progenitor cells. *Blood* 2002; 100: 3527–35.

30. Trojan L, Thomas D, Friedrich D et al. Expression of different vascular endothelial markers in prostate cancer and BPH tissue: an immunohistochemical and clinical evaluation. *Anticancer Res* 2004; 24: 1651–6.

31. Lackner C, Jukic Z, Tsybrovskyy O et al. Prognostic relevance of tumour-associated macrophages and von Willebrand factor-positive microvessels in colorectal cancer. *Virchows Arch* 2004; 445: 160–7.

32. De Raeve H, Van Marck E, Van Camp B et al. Angiogenesis and the role of bone marrow endothelial cells in haematological malignancies. *Histol Histopathol* 2004; 19: 935–50.

33. George F, Brisson C, Poncelet P et al. Rapid isolation of human endothelial cells from whole blood using S-Endo-1 monoclonal antibody coupled to im-

muno-magnetic beads: demonstration of endothelial injury after angioplasty. *Thromb Haemost* 1992; 67: 147–53.

34. Blann AD, Woywodt A, Bertolini F et al. Circulating endothelial cells: Biomarker of vascular disease. *Thromb Haemost* 2005; 93: 228–35.

35. McClung JA, Naseer N, Saleem M et al. Circulating endothelial cells are elevated in patients with type 2 diabetes mellitus independently of HbA(1)c. *Diabetologia* 2005; 48: 345–50.

36. Solovey A, Gui L, Key NS et al. Tissue factor expression by endothelial cells in sickle cell anaemia. *J Clin Invest* 1998; 101: 1899–904.

37. Wagner DD. Cell biology of von Willebrand factor. *Ann Rev Cell Biol* 1990; 6: 217–46.