Circulating Soluble E-Cadherin Levels are of Prognostic Significance in Patients with Multiple Myeloma

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Abstract. *Background: The epithelial transmembrane molecule E-cadherin (E-Cad) is the prime mediator of epithelial cell-cell adhesion, through homotypic interactions. It also participates in the maintenance of cytoskeletal structure and cell-cell signalling, while there are no published reports of expression of E-Cad in non-epithelial tissues. We examined whether the circulating levels of soluble E-Cad in newly diagnosed patients with multiple myeloma (MM) are of prognostic significance. Patients and Methods: We used an ELISA method to determine the levels of circulating soluble E-cadherin (sE-Cad) in 21 newly diagnosed patients with MM and in 29 healthy volunteers, as a control group. Results: MM patients demonstrated increased circulating levels of sE-Cad, compared with controls ($p<0.0001$). Increased circulating sE-Cad levels correlated with LDH levels at diagnosis ($p<0.001$) and poor prognosis. Multivariate analysis demonstrated that sE-Cad levels are an independent prognostic factor of survival ($p<0.0207$). Conclusion: Our data suggest that adhesion molecules play a role in the pathogenesis of MM, establish sE-Cad as an independent marker of survival and, finally, provide evidence of non-epithelial production of E-Cad in MM patients.*

Abbreviations. BM, bone marrow; BM SCs, bone marrow stromal cells; CEA, carcinoembryonic antigen; E-Cad, E-cadherin (epithelial-cadherin); ICAM, intercellular adhesion molecule; MM, multiple myeloma; NCAM, neural cell adhesion molecule; PECAM, platelet epithelial cell adhesion molecule; PCL, plasma cell leukemia; sE-Cad, soluble E-cadherin; TNF-α, tumor necrosis factor-alpha; TGF-β, transforming growth factor-beta; VCAM, vascular cell adhesion molecule; VLA, very late antigen.

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Key Words: Adhesion, E-cadherin, prognosis, multiple myeloma.

Multiple Myeloma (MM) is a B-cell disease, characterized by a monoclonal expansion of plasma cells localized in the bone marrow (BM) and the peripheral blood, which express the same immunoglobulin (Ig) gene rearrangements (1-3). Although plasma cells represent the dominant malignant cell type in MM, it is now accepted that more immature B-cells, most likely from germinal centre B-cells, are also part of the neoplastic cell clone (3-5). In fact, it has been suggested that these cells might migrate from the lymph nodes towards the BM environment, where they proliferate and differentiate into mature plasma cells under the appropriate stimuli (3,6). The exact mechanisms by which myeloma cells localize and interact with the BM environment have not been elucidated yet, but it is clear that adhesion molecules play a pivotal role in this process (3,7,8).

MM cells specifically adhere to the extracellular matrix and the bone marrow stromal cells (BM-SCs) *via* very late antigen-4 (VLA-4), vascular cell adhesion molecule - 1 (VCAM-1), intercellular adhesion molecule–1 (ICAM-1) and CD44. These intercellular interactions between MM cells and BM-SCs have been implicated in the growth, differentiation and survival of MM cells, as well as the progression of the disease, *via* the production of cytokines, mainly of IL-6 (8-10). In MM patients, IL-6 plays an important role in bone remodelling by regulating skeletal homeostasis: it stimulates the recruitment of osteoclasts, while it has been shown recently that it can also promote the proliferation of osteoblasts (4,5,11,12). After adhering to BM-SCs, MM cells grow and survive at least in part due to autocrine and paracrine signalling by IL-6. Moreover, increased expression of adhesion molecules by MM cells localized to bone marrow triggers the production of TGF-β1. MM cells, in contrast to normal B-cells, lack receptors for TGF-β1 and, therefore, are not down-regulated by its increased levels.

The high local concentrations of TGF-β1, however, promote the production of cytokines, including tumour necrosis factor - α (TNF-α) and IL-6 (8,12,14). In addition to contributing to the growth and proliferation of MM cells, IL-6 also inhibits their apoptosis and affects the expression of adhesion molecules by up-regulating type I collagen and down-regulating syndecan-1.

As the disease progresses, this facilitates mobilization of MM cells from the bone marrow and promotes cell spread and development of plasma cell leukaemia (PCL). Away from the bone marrow microenvironment and the influence of IL-6, PCL cells regain syndecan-1 expression and bind to metastatic sites (8,13,15).

Cadherins are a family of transmembrane glycoproteins with an adhesive role: they mediate homophilic Ca^{2+}-dependent cell-cell adhesion. They have been shown to play a crucial role in cell migration and tissue morphogenesis, while they also participate in wound healing and cell migration. E-Cad is a cell-adhesive molecule found in epithelial cells in a variety of embryonic and adult tissues. Several recent studies have suggested a link between unstable or reduced expression of E-Cad and tumour progression in solid neoplasms (16-20). Soluble forms of E-Cad are found as 80-84 kDa peptides released from the human carcinoma cell line (MCF-7) into the culture medium. These fragments are degradation products of the 120 kDa form of the intact E-Cad. They disrupt intercellular connections in cultural epithelial cells and they have been shown to be a good indicator of the regeneration of E-Cad *in vivo* (21-24). Although the expression of E-Cad in solid malignancies has been thoroughly examined, little is known about its expression in haematological malignancies.

IL-6 is known to promote the proliferation and metastatic potential of cancer cells in solid tumours. This involvement of IL-6 at a cellular level with the processes of cancer control is reflected by the results of serum studies of cancer patients, where IL-6 may reflect prognosis and tumour load. Thus it has been recently reported that patients with metastatic ovarian, lung and renal cell carcinomas have higher serum IL-6 levels than those without disseminated disease (9,10). It has also been demonstrated that elevated IL-6 levels are associated with a poor prognosis in tumours such as non-small cell lung cancer, although the ontological role of IL-6 in this process is not known (11).

In the present study, we investigated the levels of soluble E-cadherin (sE-Cad) and of IL-6 in patients with MM and we correlated them with the clinical and pathological parameters of the disease.

Patients and Methods

Subjects. Our study included two groups (Table I). The subjects comprised twenty-one consecutive, newly diagnosed patients with MM, treated at the Oncology/Haematology Unit, 3rd Department of Medicine, Athens Medical School, Sotiria General Hospital, Greece. These included 10 males and 11 females, aged 42-78 years (mean age 68.24 yrs). The patients were diagnosed and staged according to the system suggested by Durie and Salmon (25). Our study group consisted of stage I: 6 patients; stage II: 8 patients; stage III: 7 patients. The patients were followed for 5 years (median 3.7 yrs; range 1.0-5.7 yrs). Three patients were lost to follow-up. Twenty-nine apparently healthy volunteers were used as a control group (mean age 69.54 yrs; range 19-68 yrs).

Table I. *Baseline characteristics of multiple myeloma patients and healthy individuals (controls).*

Characteristics		Number (%)
PATIENTS (n = 21)		
Gender: male		10
	female	11
Age (years)		68.2 (8)*
Stage	1	6 (29%)
	2	8 (38%)
	3	7 (33 %)
E-Cad (ng/mL)		3291.4 (740-8331)**
E-Cad (ng/mL)		3973.2 (2407.1)*
IL-6 (pg/mL)		10.6 (6.5-27.8)**
LDH		240.7 (72.48)*
CONTROLS (n = 29)		
Gender: male		17
	female	12
Age (years)		69.54 (9.3)*
E-Cad (ng/mL)		648.5 (360)*
IL-6 (pg/mL)		10.4 (5.5-27.3)**

* mean (SD)
** median (min, max)

Methods. All serum samples were prospectively collected with the appropriate Ethical Committee permissions. Each sample had been collected in a Vacutainer (Becton Dickinson, Plymouth, UK) and, after spinning for 15 minutes at 1500g at room temperature, was stored without preservative at -80˚C and blindly tested with the application of an immunoenzymatic method.

Serum E-cadherin concentrations were determined using the commercially available enzyme-linked immuno-sorbent assay (ELISA) kit supplied by Takara Shuzo Co, Ltd (Ozaka, Japan). Serum IL-6 concentrations were determined using the commercially available enzyme-linked immuno-sorbent assay (ELISA) kit supplied by Biosource International, (California, USA). Both assays employ the quantitative sandwich enzyme immunoassay technique using recombinant human IL-6 and serum E-cadherin with antibodies raised against the recombinant proteins respectively.

Optical density was read with a microtiter plate reader by dual wavelength at 450 nm. All samples were assayed in duplicate.

Statistical analysis. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software. Comparisons of the sE-Cad and IL-6 levels between MM patients and controls were performed with the Wilcoxon Rank Sum test because their distributions deviated from normality. A cut-off point of twice the highest sE-Cad value from the healthy subjects' group was used to divide the MM patients into two groups with "high" and "low" sE-Cad values. The survival distribution functions for those two groups were estimated with the Kaplan Meier method and their equality was tested with the log rank test.

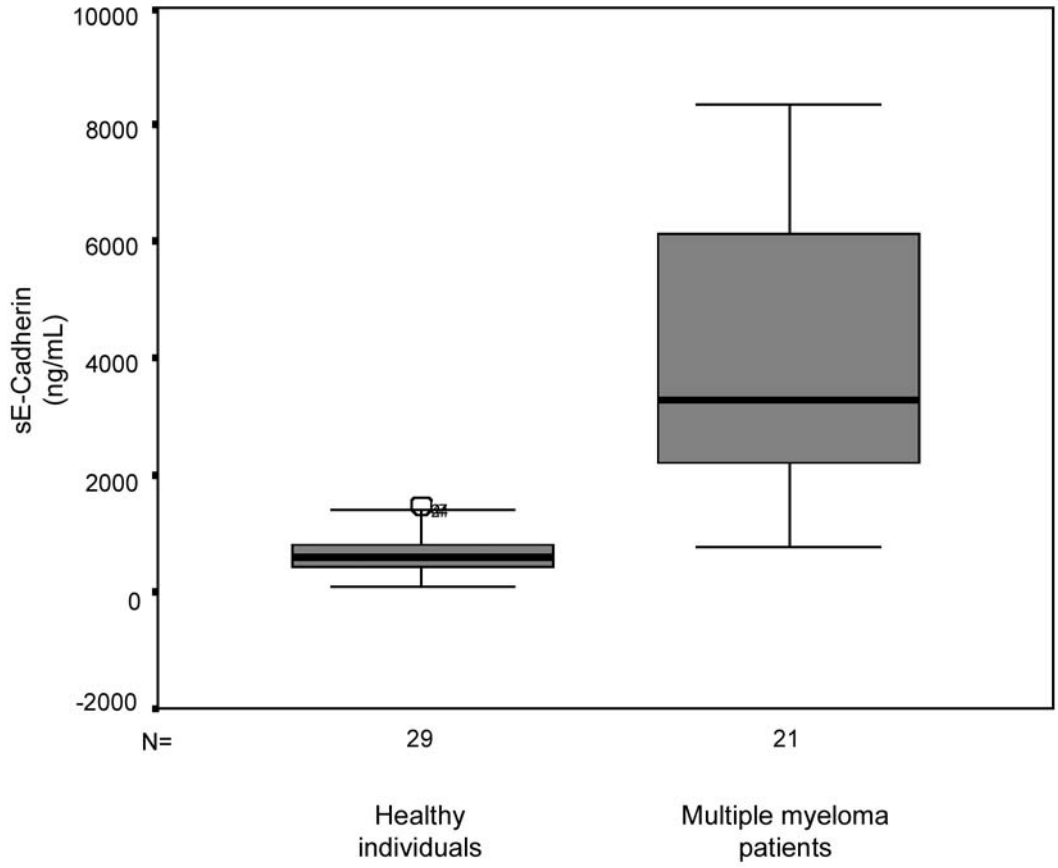

Figure 1. *The distributions (box-plots: medians, quartiles and extreme values) of sE-Cad values among MM patients and healthy controls.*

Multivariate survival analysis for MM patients was performed with Cox proportional hazards models. The association of sE-Cad (continuous, in increments of 100 ng/ml) and other variables such as IL-6 (continuous) and LDH (continuous) with death from MM was examined by alternatively introducing them in a multivariate model, adjusting simultaneously for age (continuous), gender and stage (in 3 groups as shown in Table I, ordinal).

Results

The characteristics of patients and controls can be seen in Table I. The median follow-up period was 3.7 years (range: 1-5 yrs). Three patients were lost in follow-up and there were 11 (52.4%) deaths in that period.

Circulating sE-Cad levels were found to be significantly increased in MM patients, compared with the control group, with median values of 3291.4 ng/ml and 622.9 ng/ml, respectively. The difference was highly statistically significant ($p<0.0001$; Wilcoxon Rank Sum test). The distributions (box-plots) of sE-Cad values among MM patients and controls are shown in Figure 1. There was no significant difference in the sE-Cad values between males and females both in the patients and the control group ($p=0.1$ and $p=0.28$, respectively, Wilcoxon Rank Sum test).

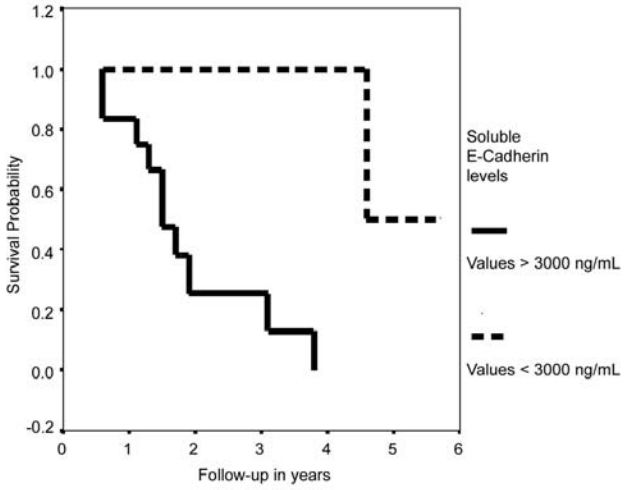

Figure 2. *MM patients were divided into two groups according to the values of sE-Cad, with cut-off point at 3000 ng/mL, which equals two times the highest value observed in the healthy subjects group. The Kaplan-Meier curves illustrating the survival functions for the two groups demonstrate that the difference between the two groups is highly significant ($p<0.0015$, log rank test).*

Table II. *Hazard ratios (and 95% confidence intervals) expressing the association of sE-Cad, LDH and IL-6 with mortality in 21 multiple myeloma patients, after alternative introduction in a model adjusting simultaneously for age, gender and disease stage.*

Variables	Hazard ratio	95% CI	*p*-value
Age (years)*	1.035	0.93, 1.16	0.54
Gender (male)*	3.057	0.46, 20.18	0.25
Stage (ordinal)*	1.226	0.36, 4.232	0.75
sE-Cad	1.057	1.01, 1.1	0.013
LDH (mg/dl)	1.007	0.998, 1.02	0.137
IL-6 (pg/mL)	0.964	0.83, 1.11	0.62

*HRs, 95% CIs, and *p*-values from the model with sE-Cadherin

Comparisons of IL-6 levels between MM patients and controls showed no significant difference between the two groups ($p=0.6$, Wilcoxon Rank Sum test, Table II).

In an attempt to divide the patients into two groups (high and low), according to the values of sE-Cad, we chose a cut-off point of 3000 ng/ml, which equals two times the highest value observed in the healthy subjects group. Eight out of the 21 patients (8/21, 38%) were below this cut-off point and the Kaplan Meier curves illustrating the survival functions for the two groups can be seen in Figure 2. The difference between the two survival distribution functions was highly significant ($p=0.0015$, log rank test).

In the multivariate analysis only sE-Cad was identified as a significant predictor of mortality, at the $p=0.05$ level, while increasing age, LDH levels, escalated disease stage and male gender were positively but not significantly associated with the endpoint (Table II). An increase of 100 ng/ml in sE-Cad was independently associated with almost a 6% increase in the risk of dying for the MM patients (Hazard Ratio: 1.057, 95% CIs: 1.012, 1.105; $p=0.013$).

Discussion

Extensive evidence, produced during the last few years, has elucidated a strong link between the localisation of myeloma cells to the BM and the pathogenesis of MM. Cellular contact between myeloma cells and the BM stroma triggers several paracrine and autocrine mechanisms, which regulate the proliferation and differentiation of myeloma cells. Therefore, knowledge of the expression of the adhesion molecules involved in these interactions is essential in understanding the disease process (8). Several investigators have reported enhanced expression of adhesion molecules, such as NCAM, ICAM-1, VLA-4, CD44 and CD56. The expression of these adhesion molecules is regulated by cytokines, while adhesion itself can trigger cytokine secretion (mainly IL-6 and TGF-β), which further augments expression of adhesion molecules (26-28). The adhesion molecules studied up to now in MM patients mainly regulate cell-to-substrate interactions (28), while there is no information regarding the expression of E-Cad, the main intercellular adhesion molecule. This member of the cadherin family regulates Ca^{2+}-dependent interactions between cells of epithelial origin.

It is now recognized that abnormal tissue expression of E-Cad is associated with increased invasiveness, enhanced metastatic potential and poor prognosis in patients with epithelial malignancies. Recent immunohistochemical findings suggest that loss of normal membranous E-Cad expression is correlated with a more aggressive phenotype in various epithelial malignancies, such as colorectal, gastric, pancreatic, lung and ovarian cancers (29-31). Furthermore, some reports have demonstrated increased serum concentrations of soluble E-cadherin in various epithelial malignancies, such as colorectal, gastric, lung and bladder cancer, with uncertain biological significance.

Finally, in addition to serum, fragments of soluble E-Cad can be detected in several human body fluids such as citrated plasma, urine, normal amniotic and bullous pemphigoid blister fluid samples (32-35).

Our study is the first to demonstrate significantly increased (up to 6-fold) levels of sE-Cad in the serum of patients newly diagnosed with multiple myeloma. Furthermore, when compared to healthy controls, this increase is statistically highly significant ($p<0.0001$). On the contrary, no correlation between increased serum levels of sE-Cad and advanced stage of disease was established. This observation could be an indication that sE-Cad levels increase early in the progress of the disease and, therefore, play a crucial role in the pathogenesis of the disease. On the other hand the relatively small number of patients studied (although evenly distributed per stage) could be the reason for this observation. With regard to the origin of serum sE-Cad, it is possible that its levels reflect an increased rate of turnover of tissue E-Cad, or acquired genetic abnormalities.

According to our findings increased levels of sE-Cad were of prognostic value, since patients with sE-Cad serum values >3000 ng/mL had a worse outcome, compared with patients with sE-Cad serum values <3000 ng/mL. It is also important that, in the multivariate analysis performed, sE-Cad levels were an independent factor in predicting patients' survival.

Our results, if confirmed by larger studies, could form the basis for the establishment of serum sE-Cad level at diagnosis, as a predictor of overall survival of MM patients. In conclusion, we demonstrated, for the first time, significantly increased serum levels of sE-Cad in patients with MM, a disease of non-epithelial origin. It seems that serum sE-Cad increases early in the progress of MM and its level at diagnosis is an independent, negative predictor of overall survival. A larger study correlating

sE-Cad levels with immunohistochemistry of bone marrow biopsy specimens is currently being undertaken to clarify the role of E-Cad in the pathogenesis of this disease.

References

1 Feinman R, Sawyer J, Hardin J and Tricot G: Cytogenetics and molecular genetics in Multiple Myeloma. Hematol/Oncol Clin North Am *11*: 1-25, 1997

2 Faid L, Van Riet I, de Waele M *et al*: Adhesive interactions between tumor cells and bone marrow stromal elements in human multiple myeloma. Eur J Haematol *57*: 349-358, 1996.

3 Bakkus MHC, Heirman C, Van Reit I, Van Camp B and Thielemans K: Evidence that multiple myeloma Ig heavy chain VDJ genes contain somatic mutations but show no intraclonal variation. Blood *80*: 2326-2335, 1992.

4 Klein B and Bataille R: Cytokine network in human multiple myeloma. Hematol/Oncol Clin. North Am *6*: 273-284, 1992.

5 Klein B: Cytokine, cytokine receptors, transduction signals, and oncogenes in human multiple myeloma. Semin Hematol *32*: 4-19, 1995.

6 Merico F, Bergui L, Gregoretti MG *et al*: Cytokines involved in the progression of multiple myeloma. Clin Exp Immunol *92*: 27-31, 1993.

7 Van Riet I, De Waele M, Remels L, Lacor P, Schots R and Van Camp B: Expression of cytoadhesion molecules by myeloma plasma cells. Br J Haematol *79*: 421-427, 1991.

8 Teoh G and Anderson K: Interaction of tumor and host cells with adhesion and extracellular matrix molecules in the development of multiple myeloma. Hematol/Oncol Clin North Am *11*: 27-42, 1997.

9 Stauder R, Van Driel M, Schwarzler C *et al*: Different CD44 splicing patterns define prognostic subgroups in multiple myeloma. Blood *88*: 3103-3108, 1996.

10 Ashman EJ, Lockhorst HM, Dekker AW and Bloem AC: Lymphocyte function-associated antigen-1 expression on plasma cells correlates with tumor growth in multiple myeloma. Blood *79*: 2068-2075, 1992.

11 Urashima M, Chauhan D, Uchiyama H, Freeman GJ and Anderson KC: CD40 ligand triggered IL-6 secretion in multiple myeloma. Blood *85*: 1903-1912, 1995.

12 Urashima M, Ogata A, Chauhan D *et al*: Transforming growth factor-beta1: Differential effects on multiple myeloma *versus* normal B cells. Blood *87*: 1928-1938, 1996.

13 Urashima M, Ogata A, Chauhan D *et al*: IL-6 promotes multiple myeloma growth *via* phosphorylation of retinoblastoma protein. Blood *88*: 2219-2227, 1996.

14 Uchiyama H, Barut BA, Mohrbacher AF, Chauhan D and Anderson KC: Adhesion of human myeloma-derived cell lines to bone marrow stromal cells stimulates IL-6 secretion. Blood *82*: 3712-3720, 1993.

15 Vidriales MB and Anderson KC: Adhesion of multiple myeloma cells to the bone marrow microenvironment. Implications for future therapeutic strategies. Mol Medic Today *2*: 425-431, 1996.

16 Silye R, Karayiannakis AJ, Syrigos KN *et al*: E-cadherin/catenin complex in benign and malignant melanocytic lesions. J Pathol *186*: 350-355, 1998.

17 Karatzas G, Karayiannakis AJ, Syrigos KN *et al*: E-cadherin expression correlates with tumour differentiation in colorectal cancer. HepatoGastroenterology *46*: 232-235, 1999.

18 Seery JP, Syrigos KN, Karayiannakis AJ, Valizadeh A and Pignatelli M: Abnormal expression of the E-cadherin-catenin complex in dysplastic Barrett's oesophagus. Acta Oncologica *38*: 945-948, 1999.

19 Karatzas G, Karayiannakis AJ, Syrigos KN *et al*: Expression patterns of the E-cadherin-catenin cell-cell adhesion complex in gastric cancer. HepatoGastroenterology *47*: 1-4, 2000.

20 Karayiannakis AJ, Syrigos KN, Chatzigianni E *et al*: Aberrant E-cadherin expression associated with loss of differentiation and advanced stage in human pancreatic cancer. Anticancer Res *18*: 4177-4180, 1998.

21 Damsky CH, Richa J, Solter D, Knudsen K and Buck CA: Identification and purification of a cell surface glycoprotein mediating intercellular adhesion in embryonic and adult tissue. Cell *34*: 455, 1983.

22 Wheelock MJ, Buch CA, Bechtol KB and Damsky CH: Soluble 80-kD fragment of cell-CAM 120/80 disrupts cell-cell adhesion. J. Cell Biochem *28*: 217-224, 1987.

23 Syrigos KN, Harrington KJ and Pignatelli M: Role of adhesion molecules in bladder cancer: an important part of the jigsaw. Urology *53*: 428-434, 1999.

24 Nemunaitis J, Andrews DF, Mochizuki DY, Lilly MB and Singer JW: Human marrow stromal cells: Response to IL-6 and control of IL-6 expression. Blood *74*: 1929-1935, 1989.

25 Durie BGM and Salmon SE: A clinical staging system for multiple myeloma. Correlation of measured myeloma cell mass with presenting clinical features, response to treatment and survival. Cancer *36*: 842-854, 1975.

26 Ong F, Kaiser U, Seelen PG *et al*: Serum neural cell adhesion molecule differentiates multiple myeloma from paraproteinemias due to other causes. Blood *87*: 712-716, 1996.

27 Patric JrCW, Juneja HS, Lee S, Schmalstieg FC and McLative LV: Heterotypic adherence between human B-lymphoblastic and pre-B-lymphoblastic cells and marrow stromal cells is a biphasic event: Integrin very late antigen-4a mediates only the early phase of the heterotypic adhesion. Blood *85*: 168-178, 1995.

28 Pellat-Deceunynck C, Barille S, Puthier D *et al*: Adhesion molecules on human myeloma cells: Significant changes in expression related to malignancy, tumor spreading and immortalization. Cancer Res *55*: 3647-3653, 1995.

29 Karayiannakis AJ, Syrigos KN, Efstathiou J *et al*: Expression of catenins and E-cadherin during epithelial restitution in inflammatory bowel disease. J Pathol *185*: 413-418, 1998.

30 Syrigos KN, Krausz T, Waxman J *et al*: E-cadherin expression in bladder cancer using formalin-fixed, paraffin-embedded tissues: correlation with histopathological grade, tumour stage and survival. Int J Cancer *64*: 367-370, 1995.

31 Velicova G, Banks RE, Gearing A *et al*: Serum concentrations of soluble adhesion molecules in patients with colorectal cancer. Br J Cancer *77*: 1857-1863, 1998.

32 Banks RE, Porter WH, Whelan P, Smith PH and Selby PJ: Soluble forms of the adhesion molecule E-cadherin in urine. Human Pathol *48*: 179-180, 1995.

33 Velicova G, Banks RE, Gearing A *et al*: Circulating soluble adhesion E-cadherin, E-selectin, ICAM-1 and VCAM-1 in patients with gastric cancer. Br J Cancer *76*: 1398-1404, 1997.

34 Griffiths TRL, Brotherick I, Bishop RI *et al*: Cell adhesion molecules in bladder cancer: soluble serum E-cadherin correlates with predictors of recurrence. Br J Cancer *74*: 579-584, 1996.

35 Pittard AJ, Banks RE, Galley HF and Webster NR: Soluble E-cadherin concentrations in patients with systemic inflammatory response syndrome and multiorgan dysfunction syndrome. Br J Anaesthesia *76*: 629-631, 1996.

Received December 8, 2003
Accepted February 10, 2004