A standardized endogenous megakaryocytic erythroid colony assay for the diagnosis of essential thrombocythemia

[haematologica]
2004;89:1207-1212

IRÈNE DOBO
NATHALIE BOIRET
ERIC LIPPERT
FRANÇOIS GIRODON
PASCAL MOSSUZ
MAGALI DONNARD
LYDIA CAMPOS
DANIELLE PINEAU
ELISABETH BASCANS
VINCENT PRALORAN
SYLVIE HERMOUET

A B S T R A C T

Background and Objectives. The reliability of assays of endogenous megakaryocytic colony (EMC) and endogenous erythroid colony (EEC) formation for the diagnosis of thrombocytoses remains controversial. We tested the suitability of a recently developed collagen-based assay of EMC formation for the diagnosis of essential thrombocythemia (ET).

Design and Methods. This was a multicenter (8 laboratories) study including 121 patients: 82 with ET and 39 with reactive thrombocytoses (RT). EMC and EEC were assessed in each laboratory in serum-free, cytokine-free, standardized collagen gel assays; bone marrow (BM) and peripheral blood (PB) were tested in parallel.

Results. In PB cultures, only EEC were specific for ET. In BM cultures, both EMC and EEC were specific for ET and present in assays of 77.8% (EMC) and 33.3% (EEC) of ET patients. Altogether, 80.2% of ET patients had BM EMC and/or EEC, whereas none of the patients with RT did.

Interpretation and Conclusions. When performed with BM progenitors for the diagnosis of thrombocytoses, positivity of the standardized EMC/EEC assay in collagen is specific (100%) and detects 80% of ET.

Keywords: endogenous megakaryocytic colony formation, endogenous erythroid colony formation, essential thrombocythemia, collagen, standardization.

From the Laboratoires d' Hématologie of the Centres Hospitaliers Universitaires d'Angers (ID), Clermont-Ferrand (NB), Bordeaux (EL), Dijon (FG), Grenoble (PM), Limoges (MD), Saint-Etienne (LC), Nantes (DP, SH), France.

Correspondence: Dr. Sylvie Hermouet, Laboratoire d'Hematologie, Institut de Biologie, CHU Nantes, 9 quai Moncousu, 44093 Nantes cedex, France. E-mail: sylvie.hermouet@chu-nantes.fr

@2004, Ferrata Storti Foundation

©Ferrata Storti Foundation

Essential thrombocythemia (ET) is characterized by chronically raised platelet counts. According to the Polycythemia Vera (PV) Study Group (PVSG) and the World Health Organization (WHO),[1,2] a diagnosis of ET is made on two positive criteria (platelets > 600×10^9/L, bone marrow (BM) histology in favor of ET) and exclusion of other causes of thrombocytosis: reactive thrombocytosis (RT), other myeloproliferative disorders (MPD) (PV, chronic myeloid leukemia, idiopathic myelofibrosis), or myelodysplastic syndromes. No additional test can facilitate the diagnosis of ET. Evidence of X-linked clonality can be useful but it is not a criterion for ET for several reasons: it is limited to females; some cases of ET are oligoclonal or polyclonal; monoclonality can be found in healthy older females.[3-6] Similarly, because of its problematic interpretation, partly due to the absence of standardization,[2,7-9] the endogenous colony assay is not a criterion for diagnosing ET. Yet formation of endogenous megakaryocytic colonies (EMC) and endogenous erythroid colonies (EEC) is well described in ET. EEC, specific to MPD, are clearly in favor of the diagnosis of ET but are present in only a minority of ET patients.[6,10-16] The specificity and diagnostic value of EMC, depending on culture conditions in plasma clot or methylcellulose, with BM or peripheral blood (PB) cells, remain controversial.[8,9,11,17-21]

We recently described serum-free, cytokine-free collagen-based assays with improved detection of EEC and EMC due to *in situ* colony staining.[14,15,17,22,23] The collagen EEC assay now standardized for the diagnosis of PV is specific (100%) and sensitive (83%).[15,22] Preliminary studies indicated that the collagen assay allows formation by BM progenitors of EMC which could be specif-

Table 1. Diagnostic criteria.

Diagnostic	*Criteria*
Essential Thrombocythemia (ET)	WHO ET criteria Platelet counts: > 600×10^9/L. Characteristic changes in bone marrow histology: increased numbers ofmegakaryocytes, enlarged with multilobulated nuclei, clustered in small groups; increase in reticulin, or myelofibrosis. Exclusion of other causes of thrombocytosis: RT, myeloproliferative disorders other than ET (polycythemia vera, chronic myeloid leukemia, idiopathic myelofibrosis) and myelodysplastic syndromes.
Reactive Thrombocytosis (RT)	Platelet counts: > 600×10^9/L. All patients were recovering from recent surgery. All had normal platelet counts prior to surgery and it was verified that platelet counts came back to normal after surgery (usually within weeks, occasionally within months of surgery).

ic to ET.[17,22] The present multicenter study tested the value of a standardized collagen EMC/EEC assay for the diagnosis of ET. Because PB EMC formation also occurs in ET,[21,24,25] we compared the use of BM and PB for the EMC/EEC assay.

Design and Methods

Patients

BM and PB samples were obtained from 121 patients diagnosed in 16 hospitals as having RT (n=39) and ET (n=82) using the WHO ET criteria[2] (Table 1). Samples collected in clinics far from the 8 participating laboratories were sent by overnight delivery.

Cultures

BM and PB mononuclear cells (BMMC and PBMC) were prepared as described previously.[22] Fresh BM and PB samples were centrifuged at 100 g for 10 min continuously at room temperature. Platelet-rich supernatants were removed, nucleated cells were carefully pipetted from the surface of the red blood cell pellet, suspended in Hank's buffer and separated on Ficoll medium (1.077 g) by centrifugation at 400 g for 30 min continuously at room temperature. In order to eliminate platelets, cells were washed once with Hank's buffer without calcium or magnesium, once with PBS + 1% EDTA at 4°C and once in Iscove's medium. For the EMC/EEC assay, BMMC (10^5/mL) and PBMC (2×10^5/mL) were then plated in 1 mL of collagen-based semisolid medium without serum or cytokines (medium 1: MegaCult with lipids and human collagen (StemCell Technologies, Meylan, France; catalog references 4850 and 4802) in triplicate (330 µL/well) in 4-well plates (Nunc-Ruskilde, Denmark) and grown for 10 days. As controls of progenitor growth, BFU-E and CFU-MK assays were performed in the same serum-free, collagen-based medium supplemented with appropriate cytokines: medium 2 for BFU-E assays contained 50 ng/mL stem cell factor, 10 ng/mL interleukin-3 (IL-3), 10 ng/mL IL-6, 10 ng/mL granulocyte-macrophage colony stimulating factor (GM-CSF), 10 ng/mL G-CSF and 3 U/mL erythropoietin (EPO); medium 3 for CFU-MK assays contained 10 ng/mL IL-6, 10 ng/mL IL-3 and 50 ng/mL thrombopoietin. In BFU-E assays, 2×10^4 BMMC/mL or 5×10^4 PBMC/mL were plated, whereas for the CFU-MK assays, 10^5 BMMC/mL or 2×10^5 PBMC/mL were plated. BFU-E were counted on day 14, CFU-MK on day 10. Culture media and collagen were provided by the manufacturers. PB cultures were not performed for 7 ET patients; BM was not available for 21 RT patients. When the number of progenitors available for culture was insufficient, the control CFU-MK assay was omitted. The gels were harvested on glass slides, dried and stained with May-Grünwald-Giemsa (MGG) as described elsewhere,[15,22,23] and colonies were counted on triplicate gels. The numbers of colonies were expressed as averages per 10^5 cells. The identification of MGG-stained CFU-MK (> 4 megakaryocytes) in collagen (Figure 1) is as accurate as immunocytochemical labeling with anti-CD41/61 antibodies.[17,26] For erythroid colonies, both CFU-E (> 8 erythroblasts) and BFU-E (> 100 erythroblasts or several clusters of more than 20 erythroblasts) were counted. EMC/EEC assays were positive when CFU-MK, CFU-E or BFU-E were identified on MGG-stained gels. The EMC/EEC assay could be considered definitely negative when these CFU were not found but the number of cytokine-stimulated BFU-E in control cultures was above the threshold of $50/10^5$ BMMC or $20/10^5$ PBMC.[22] A negative BM EMC/EEC assay from 1 ET

©Ferrata Storti Foundation

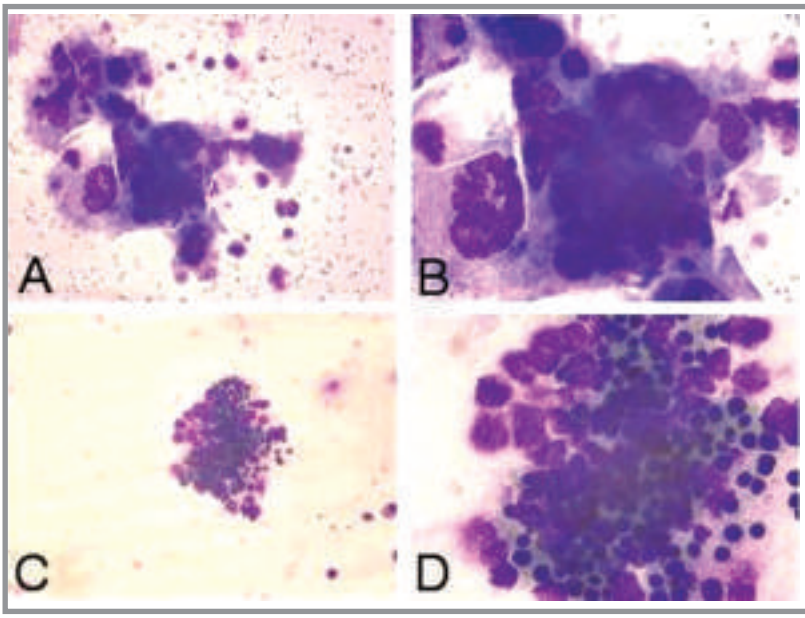

Figure 1. EMC/EEC formation by BMMC of ET patients in serum-free, cytokine-free, collagen-based media. Typical endogenous megakaryocytic (A,B) and erythroid (C,D) colonies observed at day 10 in MGG-stained collagen gels of BM cultures. EEC were usually small, well differentiated; both EMC and EEC occasionally included lysed cells. (A,C) × 10 magnification; (B,D) enlarged view of colonies A and C.

patient with a control culture containing < 50 BFU-E/10^5 cells was eliminated from the study.

Statistical analysis

Mann-Whitney's and Spearman's tests were used to compare groups, Fisher's exact test was used to compare ratios. The SPSS software was used.

Results

Control assays with cytokines (media 2 and 3)

There was no significant difference between ET and RT in cytokine-induced growth of CFU-E, BFU-E and CFU-MK in PB and BM cultures (Tables 2 and 3). CFU-MK assays frequently gave very low numbers of colonies and therefore we could not define a practical CFU-MK threshold to evaluate progenitor growth. In contrast, 98.8% (81/82) of ET and all RT BM cultures had > 50 BFU-E/10^5 BMMC. Hence, the BFU-E assay remains the best, most practical control of the growth capacity of progenitors.

Analysis of EMC formation (medium 1)

EMC in cultures of PB progenitors (Table 2) were not specific since they were found for 87.8% of ET but also 53.1% of RT patients. Although the numbers of PB EMC in RT patients was always ≤ 10/10^5 PBMC, this threshold and the PB EMC assay had no practical diagnostic value because only 23% of ET patients had > 10

Table 2. EMC and EEC formation by PB progenitors.

	Peripheral blood			
	Endogenous colonies		*Cytokine-stimulated colonies*	
Diagnostic	*EMC/10^5 cells*	*EEC/10^5 cells*	*CFU-MK/10^5 cells*	*CFU-E$^+$BFU-E/10^5 cells*
ET				
n =	74	74	35	74
mean±SD	9.1±21.5	1.8±6.4	23.8±40.9	78±68
median (range)	3.0 (0-168)	0 (0-50)	8.0 (0-341)	56 (0-1905)
nbr+ (%)	65 (87.8%)	19 (25.7%)	28 (80.0%)	73 (98.6%)
RT				
n =	32	32	20	32
mean±SD	1.3*±2.3	0	9.9±11.3	85±71
median (range)	0.5 (0-10)	0	6.0 (0-43)	72 (11-384)
nbr+ (%)	17 (53.1%)	0	18 (90.0%)	32 (100%)

For EMC/EEC assays, 2×10^5 PBMC were seeded per mL of serum-free, cytokine-free, collagen-based medium 1. Cultures were incubated for 10 days, then gels were dried, stained with MGG and CFU-MK, CFU-E and BFU-E were scored. Cytokine-stimulated assays were performed in medium 3 (CFU-MK) and in medium 2 (CFU-E, BFU-E); CFU-MK assays could not be performed for all patients. (nbr +) means "number of cultures with colonies" (ie. positive). (*) p < 0.001, Mann-Whitney's rank sum test, compared to ET patients.

©Ferrata Storti Foundation

Table 3. EMC and EEC formation by BM progenitors.

	Bone marrow			
	Endogenous colonies		*Cytokine-stimulated colonies*	
Diagnostic	*EMC/10^5 cells*	*EEC/10^5 cells*	*CFU-MK/10^5 cells*	*CFU-E+BFU-E/10^5 cells*
ET				
n =	81	81	33	81
mean±SD	10.4±15.4	10.3±31.9	42.9±36.7	247±269
median (range)	5.0 (0-84)	0 (0-207)	31 (0-251)	158 (8-1771)
nbr+ (%)	63 (77.8%)	27 (33.3%)	32 (97.0%)	81 (100%)
RT				
n =	18*	18*	3	10**
mean±SD	0	0	25.7±13.8	190±181
median (range)	0	0	15 (15-47)	112 (67-700)
nbr+ (%)	0	0	3 (100%)	10 (100%)

For EMC/EEC assays, 10^5 BMMC were seeded per ml of serum-free, cytokine-free, collagen-based medium 1. Cultures were incubated for 10 days, then gels were dried, stained with MGG and CFU-MK, CFU-E and BFU-E were scored. Cytokine-stimulated assays were performed in medium 3 (CFU-MK) and in medium 2 (CFU-E, BFU-E); CFU-MK assays could not be performed for all patients. (nbr +) means "number of cultures with colonies" (ie. positive). (*) Using the same assay, we previously reported the absence of EMC and EEC in BM cultures of 18 healthy donors.[15,17] (**) For 8 RT patients, cytokine-stimulated BM cultures showed sufficient CFU-E/BFU-E growth by direct, inverted microscopy but gels were not harvested and therefore MGG-stained colonies were not counted.

EMC/10^5 PBMC. Because of the difficulty of obtaining BM from RT patients, the number of cultures of BM progenitors performed for RT patients was smaller than that for ET patients (Table 3); nevertheless, in this series, as in previous studies using the same assay,[15,17] BM progenitors of RT patients never formed EMC. In contrast, BM EMC were present in 77.8% (63/81) of ET cultures. Hence, BM cultures confirmed the specificity (100%) of the collagen EMC assay performed for the diagnosis of ET.

Analysis of EEC formation (medium 1)

PB EEC were not formed in RT cultures and were specific to patients with ET (Table 2). However, EEC were present in only 25.7% of PB ET cultures. Cultures of BM progenitors (Table 3) confirmed that EEC formation in the collagen assay is specific to myeloproliferative syndromes.[15,22] BM progenitors of RT patients never formed EEC whereas BM EEC were present in 33.3% (27/81) of ET cultures. Altogether, 80.2% (65/81) of ET patients had EMC and/or EEC in BM cultures. The numbers of BM EMC and EEC were correlated (n = 81, r = 0.41, $p < 0.001$).

Association of BM EMC and EEC

Among ET patients with BM EMC, 39.7% (25/63) also had EEC; 11.1% (2/18) of ET patients without BM EMC had BM EEC (difference not significant). In collagen, BM EMC associated with EEC are also found in a minority of patients with PV.[17,18,22] One common hypothesis concerning this *EMC$^+$ EEC$^+$* ET is that it might be a latent form of PV.[12] In our ET series, there was no significant difference in hematocrit or serum erythropoietin level (measured at diagnosis) between EMC$^+$EEC$^+$ (n = 25) and EMC$^+$EEC$^-$ (n = 38) patients. Rather, EMC$^+$EEC$^+$ ET patients had significantly (p = 0.017) higher numbers of BM EMC than did EMC$^+$EEC$^-$ ET patients: the median number of BM EMC in *EMC$^+$EEC$^+$* ET was 13 EMC/10^5 cells whereas that in *EMC$^+$EEC$^-$* ET was 5 EMC/10^5 cells.

Discussion

We have described a simple, standardized, serum- and cytokine-free collagen EMC/EEC assay suitable for the diagnosis of thrombocytoses. This assay must be performed with BM progenitors. Using the assay on a larger series of patients, eight laboratories confirmed the finding of a preliminary, single-center study of 17 patients with thrombocytosis:[17] among patients with platelet counts higher than 600×10^9/L, a positive BM EMC assay is specific to ET. The collagen EMC/EEC assay in the ready-to-use commercial culture media described here was easily performed by all participating laboratories. In addition, since not all clinics are equipped with cell culture facilities, it is worth nothing that a 24 hr-delay did not affect the EMC/EEC assay, thus allowing shipment of BM samples to a specialized laboratory (in this study, samples were collected in 16 hospitals and sent to 8 laboratories).

As shown previously,[22] controlling the quality of progenitors, by using cytokine-stimulated cultures, is an important part of the standardized EM/EEC assay in order to eliminate false negatives. The cytokine-stimulated CFU-MK assay was not sufficiently reliable, perhaps because of the choice of cytokines in medium 3 (lack of SCF). Hence, the BFU-E assay remains the preferred control for assessing progenitor quality. As in previous studies of erythrocytoses,[22] two commercial media

©Ferrata Storti Foundation

were tested for the diagnosis of thrombocytoses; EMC and EEC formation observed in PB and BM cultures of RT and ET patients with the two media were comparable (*data not shown*).

Previous studies using the same collagen-based medium had established that BM progenitors of healthy donors and patients with secondary erythrocytosis do not form EEC or EMC (based on the observation of more than fifty BM cultures).[15,22] The sensitivity (80.2%) and specificity (100%) of the collagen BM EMC/EEC assay performed for the diagnosis of thrombocytoses were excellent and comparable to those of BM and PB EEC assays performed for the diagnosis of erythrocytoses.[15,22] In particular, the sensitivity of the assay means that additional cultures testing the sensitivity of progenitors to low doses of thrombopoietin are not necessary.[17,19,20]

Like EEC, found mainly in PV but also in ET and in idiopathic myelofibrosis with myeloid metaplasia, BM EMC in collagen assays are not exclusive to ET. Using the same commercial culture medium, we previously reported BM EMC growth in about one third of PV patients.[15,22] Interestingly, in these studies we reported six patients with absolute erythrocytosis and no BM EEC but with BM EMC (< 4 EMC/10^5 BMMC). At diagnosis, three of these patients had platelet counts over 500 000$\times 10^9$/L and BM histology, available for five patients, was in favor of ET.

The study also confirmed that EEC are seen in a minority of ET but never in RT patients; in ET, BM EEC without EMC were rare (2/81 ET patients). In our series of ET patients at diagnosis, patients with both BM EMC and EEC did not have a significantly higher hematocrit compared to those without EEC. They had high numbers of EMC, which would suggest a more severe (advanced) form of ET rather than a latent form of PV.[12] However, one cannot exclude that overt PV might appear several years after diagnosis and long term follow-up of *EMC$^+$EEC$^+$* vs. *EMC$^+$EEC$^-$* ET patients is necessary in order to draw definitive conclusions. Alternatively, compared to *BM EEC only* PV and *BM EMC only* ET, *BM EMC$^+$EEC$^+$* patients might define MPD subsets originating from a pool of early progenitors common to erythroid and megakaryocytic lineages (CFU-Meg, CFU-GEMM); this would be consistent with the variable presentation of *EMC$^+$EEC$^+$* patients (polyglobulia and/or thrombocytosis). Besides the EMC assay, more detailed and standardized evaluation of BM histology, as well as several recent molecular assays (clonality assays, PRV-1 mRNA expression assays),[16,21] should be evaluated for their relevance to the diagnosis of ET in large, multicenter series; furthermore, the results of these assays should be compared to those of the BM EMC/EEC assay. In this regard, our multicenter group has begun systematic measurements of *c-mpl* expression in platelets and *PRV-1* mRNA expression in neutrophils of patients with thrombocytosis, in parallel with the BM EMC/EEC assay. Also, although we limited our study to patients whose BM histology was in favor of ET, one can argue that because of the relative difficulty of establishing the diagnosis of ET using the current WHO criteria, the sensitivity of the collagen EMC/EEC assay could be over- or under-estimated, as some of the patients diagnosed as having ET in our series might not acually have had ET. Again, large multicenter studies of thrombocytic patients including several potential diagnostic assays and long-term clinical follow-up should answer these questions.

In conclusion, the present study shows, in a large series of patients, that the standardized collagen BM EMC/EEC assay is suitable for the diagnosis of thrombocytoses. The sensitivity (80.2%) of the assay for the diagnosis of ET is similar to the sensitivity of the EEC assay for the diagnosis of PV (83%).[15,22] The simplicity of this assay should encourage its use as a diagnostic test in thrombocytoses; further studies on larger series of thrombocytic patients will determine whether the standardized BM EMC assay should become a criterion for ET, as the EEC assay is for PV.

ID: designed the study, collected and analyzed the data, and wrote the paper; VP: designed the study; read and revised the manuscript; SH: designed the study and analyzed data; wrote the paper. The following authors carried out or supervised cell cultures. They are listed in decreasing order of the number of patients contributed to the study: NB, EL, FG, PM, MD, LC. These authors carried out cell cultures in their hospital: DP, EB. The authors reported no potential conflicts of interest.

We are indebted to our colleagues from the Departments of Internal Medicine and the Departments of Clinical Hematology of the Centres Hospitaliers of Angers, Bordeaux, Châlon-sur-Saône, Clermont-Ferrand, Dijon, Grenoble, Guéret, Laval, Libourne, Limoges, Lorient, Nantes, La Roche-sur-Yon, Saint-Etienne, Saumur and Vannes for providing samples. This work was supported by a grant from the Programme Hospitalier de Recherche Clinique 2001 (Région Bourgogne). We also thank Dr. Richard Garand (Nantes) for precious help with image processing.

Manuscript received April 15, 2004. Accepted July 7, 2004.

©Ferrata Storti Foundation

References

1. Michiels JJ, Juvonen E. Proposal for revised diagnostic criteria of essential thrombocythemia and polycythemia vera by the Thrombocythemia Vera Study Group. Semin Thrombosis Hemostasis 1997; 23:339-47.
2. Imbert M, Pierre R, Thiele J, Vardiman JW, Brunning RD, Flandrin G. Essential thrombocythemia. In: Jaffe ES, Harris NL, Stein H, Vardinan JW, editors. WHO classification of tumours: tumours of haematopoietic and lymphoid tissues. IARC Press, Lyon, 2001. p. 39-41.
3. el Kassar N, Hetet G, Li Y, Briere J, Grandchamp B. Clonal analysis of haemopoietic cells in essential thrombocythaemia. Br J Haematol 1995;90:131-7.
4. Harrison CN, Gale RE, Machin SJ, Linch DC. A large proportion of patients with a diagnosis of essential thrombocythemia do not have a clonal disorder and may be at lower risk of thrombotic complications. Blood 1999;93:417-24.
5. Shih LY, Lin TL, Lai CL, Dunn P, Wu JH, Wang PN, et al. Predictive values of X-chromosome inactivation patterns and clinicohematologic parameters for vas-

cular complications in female patients with essential thrombocythemia. Blood 2002;100:1596-601.
6. Liu E, Jelinek J, Pastore YD, Guan Y, Prchal JF, Prchal JT. Discrimination of polycythemias and thrombocytoses by novel, simple, accurate clonality assays and comparison with PRV-1 expression and BFU-E response to erythropoietin. Blood 2003;101:3294-301.
7. Michiels JJ, Barbui T, Finazzi G, Fuchtman SM, Kutti J, Rain JD, et al. Diagnosis and treatment of polycythemia vera and possible future study designs of PVSG. Leuk Lymphoma 2000;36:239-53.
8. Rolovic Z, Basara N, Gotic M, Sefer D, Bogdanovic A. The determination of endogenous megakaryocytic colony formation is an unequivocal test for discrimination between essential thrombocythemia and reactive thrombocytosis. Br J Haematol 1995;90:326-31.
9. Kimura H, Ishibashi T, Sato T, Matsuda S, Uchida T, Kariyone S. Megakaryocytic colony formation (CFU-Meg) in essential thrombocythemia: quantitative and qualitative abnormalities of bone marrow CFU-Meg. Am J Hematol 1987; 24:23-30.
10. Eridani S, Sawyer B , Pearson TC. Patterns of in vitro BFU-E proliferation in different forms of polycythemia and in thrombocythemia. Eur J Hematol 1987;38:363-9.
11. Juvonen E, Ikkala E, Oksanen K, Ruutu T. Megakaryocyte and erythroid colony formation in essential thrombocythemia and reactive thrombocytosis: diagnostic value and correlation to complications. Br J Haematol 1993;83:192-7.
12. Shih LY, Lee CT. Identification of masked polycythemia vera from patients with idiopathic marked thrombocytosis by endogenous erythroid colony assay. Blood 1994;83:744-8.
13. Ciaudo M, Hadjez JM, Teyssandier I, Coly E, Zittoun R, Marie JP. Prognostic and diagnostic value of endogenous erythroid colony formation in essential thrombocythemia. Hematol Cell Ther 1998; 40: 171-4.
14. Dobo I, Pineau D, Zandecki M, Hunault M, Hermouet S. Endogenous erythroid and megakaryocytic colony formation in serum-free, cytokine-free collagen gels. J Hematother Stem Cell Res 1999;8:601-7.
15. Dobo I, Mossuz P, Campos L, Girodon F, Allégraud A, Latger-Cannard V et al. Comparison of four serum-free, cytokine-free media for analysis of endogenous erythroid colony growth in polycythemia vera and essential thrombocythemia. Hematol J 2001;2:396-403.
16. Kralovics R, Buser AS, Teo SS, Coers J, Tichelli A, van der Maas AP, et al. Comparison of molecular markers in a cohort of patients with chronic myeloproliferative disorders. Blood 2003;102:1869-71.
17. Mi JQ, Blanc-Jouvan F, Wang J, Sotto MF, Cousin F, Castinel A, et al. Endogenous megakaryocytic colony formation and thrombopoietin sensitivity of megakaryocytic progenitor cells are useful to distinguish between essential thrombocythemia and reactive thrombocytosis. J Hematother Stem Cell Res 2001;10:405-9.
18. Yan L, el Kassar N, Gardin C, Brière J. Clonality assays and megakaryocyte culture techniques in essential thrombocythemia. Leuk Lymphoma 1996;22 Suppl 1:31-40.
19. Kawasaki H, Nakano T, Kohdera U, Kobayashi Y. Hypersensitivity of megakaryocyte progenitors to thrombopoietin in essential thrombocythemia. Am J Hematol 2001;68:194-7.
20. Axelrad AA, Eskinazi D, Correa PN, Amato D. Hypersensitivity of circulating progenitor cells to megakaryocyte growth and development factor (PEG-rHu MGDF) in essential thrombocythemia. Blood 2000;96:3310-21.
21. Florensa L, Besses C, Zamora L, Bellosillo B, Espinet B, Serrano S, et al. Endogenous eythroid and megakaryocytic circulating progenitors, HUMARA clonality assays, and PRV-1 expression: useful tools for diagnosis of polycythemia vera and essential thrombocythemia. Blood 2004; 103:2427-8.
22. Dobo I, Donnard M, Girodon F, Mossuz P, Boiret N, Boukhari R, et al. Standardization and comparison of endogenous erythroid colony assays performed with bone marrow or blood progenitors for the diagnosis of polycythemia vera. Hematol J 2004;5:161-7.
23. Lanotte M. Terminal differentiation of hemopoietic cell clones cultured in tridimentional collagen matrix: in situ cell morphology and enzyme histochemistry study. Biol Cell 1984;50:107-20.
24. Battegay EJ, Thomssen C, Nissen C, Gudat F, Speck B. Endogenous megakaryocyte colonies from peripheral blood in precursor cell cultures of patients with myeloproliferative disorders. Eur J Hematol 1989; 42:321-6.
25. Florenza L, Besses C, Woessner S, Solé F, Acin P, Pedro C, et al. Endogenous megakaryocyte and erythroid colony formation from blood in essential thrombocythemia. Leukemia 1995;9:271-3.
26. Hermouet S, Niaussat AE, Briec A, Pineau D, Robillard N, Bataille R, et al. Analysis of platelet recovery after autologous transplantation with G-CSF mobilized $CD34^+$ cells purified from leukapheresis products. Hematol Cell Ther 1997; 39: 317-25.

©Ferrata Storti Foundation