

Limited Flow Cytometry Panels on Bone Marrow Specimens Reduce Costs and Predict Negative Cytogenetics

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ABSTRACT

Objectives: To determine the clinical and financial impact and predictive value of a limited flow cytometry strategy in the evaluation of bone marrow specimens.

Methods: Consecutive bone marrow cases ($n = 1,242$) were reviewed following the independent, prospective application of two flow cytometry protocols: a limited marker strategy and a multimarker strategy. Combined morphologic and flow cytometry findings were also compared with cytogenetic results.

Results: A limited flow cytometry strategy did not have a negative impact on disease detection and resulted in reduced utilization and cost. In addition, negative combined morphology and flow cytometry had a 98.4% predictive value for negative cytogenetics ($P < .001$).

Conclusions: Careful initial evaluation of bone marrow specimens can markedly reduce the costs of bone marrow examination and significantly reduce the need for flow cytometric and cytogenetic studies on these samples.

Upon completion of this activity you will be able to:

- develop a limited panel strategy for flow cytometry in the laboratory.
- determine the economic benefit of a limited flow cytometry panel strategy in the laboratory.
- select limited flow cytometry panels based on bone marrow smear findings and patient history.

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Flow cytometry and cytogenetics are commonly employed in the evaluation of bone marrow samples obtained for the detection or staging of neoplastic disease, particularly hematologic malignancies.¹⁻³ In general, clinicians will order these tests, among others, at the time of bone marrow biopsy, and the receiving laboratories will either perform the tests themselves or submit samples to a reference laboratory for analysis. This algorithm often involves analyzing the bone marrow sample using 20 or more flow cytometry markers as recommended by consensus guidelines,⁴⁻¹¹ without taking into account the aspirate smear findings or clinical history.

We are unaware of any comprehensive study describing the benefits of triaging flow cytometric analysis of bone marrow samples by what we call a “limited flow cytometry strategy,” ie, limiting the number of flow cytometry markers used via an algorithmic approach.

In this study, we compared a limited flow cytometry strategy with the more commonly used multimarker strategy to determine if a limited flow cytometry strategy will result in lower marker utilization without affecting disease detection. We also determined whether a limited strategy, coupled with morphologic evaluation, can be used to reduce the need for cytogenetic analysis on bone marrow samples.

Materials and Methods

Bone Marrow Samples

Bone marrow pathology reports on samples received over a 19-month period (June 2009 through December 2010) by two laboratory units of a multihospital, nonacademic health system were reviewed by two board-certified hematopathologists (D.G.H. and B.H.K.). Cases for which no biopsy core was received were excluded from the study. A total of 1,246 cases were obtained and reviewed for clinical history, complete blood count indices, biopsy specimen adequacy, morphologic findings, flow cytometry results, and cytogenetic results. Four cases were excluded from the study due to suboptimal biopsy samples that could not be sufficiently correlated with other studies, yielding 1,242 cases for study. Cases were considered abnormal/positive if they demonstrated abnormal cellularity for age (such as hypoplastic marrows, which may be aplastic anemia or hypocellular myelodysplasia), myelodysplastic changes in any lineage, metastatic disease, lymphomatous infiltrates, leukemia, plasma cell dyscrasia, or a myeloproliferative disorder.

Bone Marrow Flow Cytometry Strategies

Either a multimarker strategy (n = 486) or a limited marker strategy (n = 756) was prospectively employed (Figure 1, Table 1, and Table 2). In a multimarker strategy, cases for which an aspirate or biopsy sample for flow

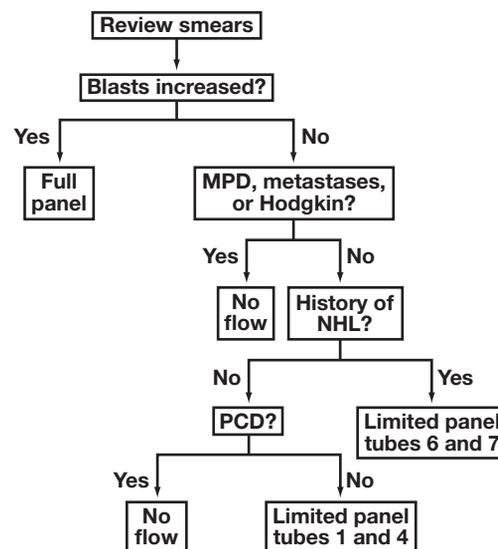


Figure 1 Limited flow cytometry strategy. Bone marrow smears are reviewed on sample receipt to determine blast percentage. Samples demonstrating no increase in blasts are directed toward the limited panel strategy pathway. Flow cytometry is not performed for staging marrows for Hodgkin lymphoma in the absence of other abnormalities, chronic myeloproliferative disorders (MPD), or in the case of plasma cell myeloma. NHL, non-Hodgkin lymphoma; PCD, plasma cell dyscrasia.

cytometry is available are analyzed when requested by the submitting physician, regardless of the smear findings.

In the limited marker strategy, bone marrow aspirate smears are prepared and in most cases reviewed within three hours of sample receipt, along with the clinical history and peripheral blood findings. Upon reviewing the aspirate smears, if the blast percentage appears to be less than 5% of nucleated cells, then a full panel is not performed. Then patient history and other smear findings are used to direct the use of a limited panel. If the bone marrow is performed for staging of Hodgkin lymphoma or classification of a chronic

Table 1
Flow Cytometry Tube Composition for the Multimarker and Limited Strategies^a

Tube No.	Marker 1	Marker 2	Marker 3	Marker 4	Marker 5	Marker 6
1	CD45	κ	λ	CD5	CD10	CD20
2	CD45	HLA-DR	CD33	CD117	CD34	C11b
3	CD45	CD16	CD64	CD14	CD13	CD11c
4	CD45	CD7	CD4	CD3	CD2	CD8
5	CD45	CD56	CD10	CD19	CD34	CD38
6	CD45	κ	λ	CD5	CD23	CD19
7	CD45	κ	λ	CD20	CD10	CD38
8	CD45	CD3	CD22	CD56	CD34	CD11c

^a The multimarker strategy consists of either tubes 1 to 5 or tubes 4 and 6 to 8. The limited strategy employed either tubes 1 and 4 or tubes 6 and 7 only. Additional markers such as CD25, CD103, cytoplasmic light chain, CD138, and immunoglobulin heavy chain classes were substituted or added in select cases at the discretion of the reviewing pathologist.

phase myeloproliferative disorder, flow cytometric analysis is not performed (Figure 1). Bone marrows for non-Hodgkin lymphoma staging or clinical paraproteinemia (monoclonal gammopathy of uncertain significance [MGUS] or unknown cause) were analyzed by a limited flow cytometry panel (tubes 6 and 7 or tubes 1 and 4; Table 1). For smears considered diagnostic for plasma cell dyscrasia (>10% plasma cells with atypia) in the appropriate clinical setting, flow cytometry was not performed in the limited strategy. Other hematologic abnormalities, such as thrombocytopenia, lymphocytosis of unknown type, and neutropenia, were evaluated by a limited strategy using tubes 1 and 4 (Table 1). Flow cytometry was not performed in cases where metastatic disease was apparent on the bone marrow smears. If an abnormal or clonal cell population was detected using the limited panel, additional markers were added, if necessary, to further classify the abnormal/clonal population. Blast percentage greater than 5% by CD45 vs side-scatter (SSC) gating also would prompt the use of additional markers to classify the blast population. Hemodiluted aspirates were not treated differently from other specimens; specimens with aparticulate smears were not analyzed by flow cytometry in the limited strategy arm.

Flow Cytometric Analysis

Heparinized bone marrow samples (or teased core biopsy specimens in select cases) were analyzed (technical component only) at Genzyme Genetics (New York, NY) Flow Cytometry Laboratory within 24 hours of collection. Immunophenotyping was performed on FACSCanto system instruments equipped with a blue 15-mW 488-nm air-cooled argon-ion laser supplemented with a 17-mW 633-nm red HeNe laser (Becton Dickinson Immunocytometry System [BDIS], San Jose, CA). Specimens were analyzed using six-color antibody panels (BDIS; Pharmingen, San Diego, CA; and Dako, Carpinteria, CA).

Flow cytometry data were analyzed using FCS Express software (DeNovo, Los Angeles, CA). A six-gate strategy was employed, using CD45 APC H7 vs SSC to characterize the lymphocyte, monocyte, granulocyte, blast, hematogone, and nucleated red cell precursor (erythroid) populations. Six- to eight-parameter analysis (forward scatter [FSC], SSC, FL1, FL2, FL3, FL4, FL5, and FL6) or multiparameter data analysis of antibody staining patterns was used to assess specific antigen expression. The analysis was interpreted by obtaining relevant data via an Internet-based portal.

Statistical analysis of the strategies was performed when relevant with an unpaired *t* test or one-sided *t* test using GraphPad software (GraphPad Software, La Jolla, CA).

Bone Marrow Cytogenetics

Bone marrow cytogenetics was performed at Genzyme Genetics. Cytogenetic specimens were cultured in T12.5 flasks, seeded with one million cells per milliliter of giant cell tumor media, 5 mL per flask. Cultures were 24-hour and 48-hour unstimulated cultures.

In most cases, 20 metaphase spreads were analyzed. Representative cells were captured using an automated imaging and karyotyping system.

Cases were reviewed by a board-certified cytogeneticist who was blinded to the morphologic and flow cytometric findings. Samples revealing only a loss of the Y chromosome were counted as normal, since this is an age-related phenomenon.

Cost Savings Analysis

Cost savings of flow cytometric analysis was determined based on our health system's technical charges per flow cytometry marker only. The cost savings of cytogenetics was based on our health system's cost for complete analysis.

Table 2
Demographics, Mean (Range) of Complete Blood Count Indices, and Marker Utilization of Cases Evaluated by the Multimarker Strategy or Limited Strategy

Characteristic	Multimarker Strategy	Limited Strategy	P Value
No. of cases	486	756	
Sex, %			
Male	50.8	54.6	
Female	49.2	45.4	
Age, mean (SD), y	60 (16.4)	62 (15.2)	.024
Hemoglobin, mean (SD), g/dL	11.6 (2.30)	11.8 (2.36)	.14
WBC, mean (SD), $\times 10^3/\mu\text{L}$	8.8 (16.4)	9.4 (19.6)	.57
Platelets, mean (SD), $\times 10^3/\mu\text{L}$	202 (160)	197 (160) ^a	.59
% Positive by flow cytometry	22.0	26.8	
No. of flow markers, mean (SD) ^b	22.0 (4.3)	11.8 (9.7)	<.001
% Flow cytometry not performed	2.3	32.5	
No. of IHC markers per case, mean (SD)	0.23 (0.77)	0.14 (0.70)	.033

IHC, immunohistochemistry.

^aPlatelet counts reported as less than $10 \times 10^3/\mu\text{L}$ were assigned a value of $10 \times 10^3/\mu\text{L}$ for computational purposes.

^bCD45 in multiple tubes was counted as one marker.

Projected national cost savings was calculated as follows:
 National cost savings = hospital system cost savings ×
 national adult population/(hospital system market size ×
 hospital system market share)

where hospital system cost savings = (difference in mean
 number markers per case between strategies × marker cost ×
 cases/y) + (cytogenetics cost × projected cases not analyzed/y).

National adult (≥18 years old) population size for 2012
 was obtained from the published national census (www.
 quickfacts.census.gov).

Limited Flow Cytometry Strategy Validation

The limited flow cytometry strategy was validated by retrospectively applying the limited strategy against specimens analyzed originally by the multimarker strategy and that displayed abnormal/clonal cell populations by flow cytometry. The original flow cytometric analysis was reviewed retrospectively after removing the data for flow cytometry markers not included in the limited strategy arm. The reported clinical history, laboratory data, and smear findings were used to guide

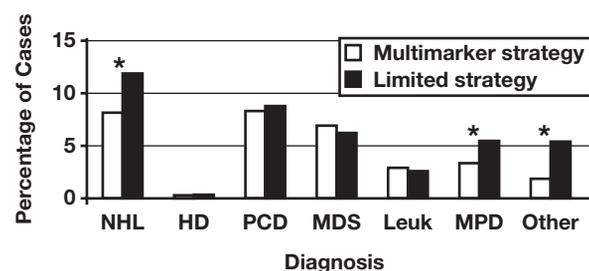


Figure 2 Disease incidence among cases evaluated by the multimarker strategy or limited strategy. HD, Hodgkin lymphoma; Leuk, acute leukemia; MDS, myelodysplastic syndrome; MPD, myeloproliferative disorder; NHL, non-Hodgkin lymphoma; PCD, plasma cell dyscrasia.
 * $P < .001$.

Table 3
 Comparison of Bone Marrow Biopsy Morphology Result With Flow Cytometry Result in Cases for Which Flow Cytometry Was Performed^a

Morphology/Flow Cytometry Result	No. (%)	
	Limited Strategy (n = 512)	Multimarker Strategy (n = 475)
+/+	120 (23.4)	90 (18.9)
+/-	66 (12.9)	55 (11.6)
-/+	17 (3.3)	13 (2.7)
-/-	309 (60.4)	317 (66.7)

+, positive; -, negative.

^a See text for description of cases with negative morphology and positive flow cytometry.

the pathway taken in the limited flow cytometry strategy. Aspirate smears were reviewed in select cases of myelodysplasia and plasma cell dyscrasia to confirm the percentage of blasts and plasma cells, respectively.

Results

Flow Cytometry

Flow cytometry interpretation was performed independently at two facilities using flow cytometry data collected via an Internet-based portal; one facility followed a multimarker strategy, and the other facility followed a limited strategy. Flow cytometry was performed in 67.7% of cases following a limited flow cytometry strategy and in 97.7% of cases following a multimarker strategy. Patient populations of the limited flow cytometry and multimarker strategy arms were similar by percent sex, mean hemoglobin, mean WBC count, and mean platelet count (Table 2). The incidence of hematologic neoplastic disease in both populations was also similar for cases of Hodgkin lymphoma, plasma cell dyscrasia, myelodysplasia, and acute leukemia; non-Hodgkin lymphoma, myeloproliferative disorders, and other disease entities were more prevalent in the limited strategy group (Figure 2). Positive cases in the limited strategy arm in which flow cytometry was not performed were predominantly due to plasma cell dyscrasia (50.5%), followed by chronic phase myeloproliferative disorders (19.8%) and metastatic carcinoma (12.9%) (data not shown).

The percentage of cases positive for neoplasia by flow cytometry using a limited strategy or multimarker strategy was similar (26.8% vs 22.0%). Overall, disease detection by both morphology and flow cytometry, if performed, was 38.4% (limited strategy) vs 30.5% (multimarker strategy). The mean number of flow cytometry markers used per case was statistically significant: 11.8 (limited strategy) vs 22.0 (multimarker strategy) ($P < .001$) (Table 2). The limited flow cytometry strategy overall was not associated with an increase in the mean number of immunohistochemical stains per case (0.14 [limited strategy] vs 0.23 [multimarker strategy]) ($P = .033$). In addition, following a limited strategy did not result in a longer reporting turnaround time, with most cases completed within one day (data not shown).

Using a limited flow cytometry strategy, flow cytometric abnormalities were detected in 17 cases (3.3% of flow cytometry samples) with no significant morphologic findings (Table 3). Thirteen cases contained small clonal B-cell populations (monoclonal B cells of uncertain significance or MBUS), three cases contained small immunoglobulin restricted plasma cell populations (MGUS), and one case contained minimal residual disease in a patient with T-cell lymphoma.

Thirteen cases (2.7% of flow cytometry samples) evaluated by the multimarker strategy displayed abnormal populations by flow cytometry where there was no significant morphologic finding: five cases with MBUS and eight cases with MGUS.

Cytogenetics

Cytogenetic analysis was performed in 453 (93.2%) of cases evaluated by the multimarker strategy and in 503 (66.5%) of cases evaluated by the limited strategy. The percentage of cases demonstrating cytogenetic abnormalities, excluding $-Y$, was similar: 14.0% for the multimarker strategy and 15.3% for the limited strategy. In cases where both morphology and flow cytometry were unrevealing, only 7 of 251 (2.8%) cases demonstrated cytogenetic abnormalities in the limited strategy arm and only 2 of 305 (0.66%) cases in the multimarker strategy arm. Of these nine cases, six displayed nonclonal abnormalities, one demonstrated a marker chromosome, and two cases displayed $t(9;22)$; these latter two cases were in patients with known chronic myelogenous leukemia who were receiving therapy. Thus combined, in those cases without a prior history of chronic myelogenous leukemia (CML) and for which both flow cytometry and morphology were negative, cytogenetic abnormalities, excluding $-Y$, were revealed in only 1.3% (7/556), and these abnormalities were not clonal; this resulted in a negative predictive value of 98.4% (Table 4). Of all cases in the study, 44.8% demonstrated no significant finding by flow cytometry or morphology.

Cost Analysis

Our system cost savings for flow cytometry using a limited strategy, based on \$14.00 per marker, was \$125,000. This translated into a projected national cost savings of \$56.4 million. Projected system savings for cytogenetics, based on the percentage of cases that could be predicted as negative, was \$185,000, with a projected national savings of \$83.3 million. Together, our flow cytometry limited strategy, coupled with reduced cytogenetic evaluation, results in a projected savings of \$310,000 for our system and \$140 million nationally.

Limited Flow Cytometry Strategy Validation

The limited flow cytometry strategy was validated by review of modified multimarker flow cytometry strategy data. The limited flow cytometry strategy, without review of aspirate or biopsy specimen slides, detected the abnormal/clonal cell population in 95 of 103 (92.2%) cases that were positive using the multimarker strategy. The remaining eight cases displayed less than 3% clonal plasma cells by flow cytometry by the multimarker strategy; seven of these cases had a clinical history of a monoclonal paraprotein and had no significant increase in plasma cells on aspirate smears or core biopsy specimen. The remaining case had a history of amyloidosis; in that case, immunohistochemistry confirmed the presence of a light chain-restricted plasma cell population.

Discussion

Flow cytometry and cytogenetic evaluation are commonly employed on bone marrow samples, regardless of the morphologic findings. Our study clearly demonstrates that a limited flow cytometry strategy using aspirate smear findings as a guide can result in a significant reduction of flow cytometry markers used and thus decrease testing costs in the hematology laboratory.

Our limited flow cytometry strategy takes advantage of evaluating bone marrow aspirates using CD45 vs SSC gating. By this method, coupled with aspirate smear morphology, the presence of an increased blast population can be easily determined, with additional markers added as necessary. To evaluate the lymphocyte population, flow cytometry markers directed toward T-cell and B-cell populations are used to exclude abnormal or neoplastic populations present in low percentages and not readily identified morphologically.

Laboratories that follow a multimarker strategy often argue that they do so because (1) they do not want to “miss” any disease not identified morphologically and (2) if a reference laboratory, they do not have the benefit of reviewing smears in advance, or the quality of the smears received

Table 4
Cytogenetics Results in Bone Marrows With Normal Morphology and Negative Flow Cytometry Evaluation in Cases Following a Multimarker or Limited Flow Cytometry Strategy

Strategy	No. of Cases	No. (%)			Negative Predictive Value, %
		Cytogenetics Normal	Cytogenetics Abnormal	Cytogenetics Not Performed	
Multimarker	317	303 (95.6)	2 (0.6)	12 (3.8)	99.3
Limited	309	244 (79.0)	7 (2.3)	58 (18.8)	97.2
Both	626	547 (87.4)	9 (1.4)	70 (11.2)	98.4

precludes adequate screening of cases. As to the first argument, in our study, disease detection rates using the limited strategy were either similar to that of the multimarker strategy or slightly higher (Figure 2). A limited flow cytometry strategy did not translate into a lower disease detection rate; in fact, overall, disease detection was higher in cases evaluated by a limited strategy (36.3% vs 30.7%) and, for some disease classes, was statistically significant. This may be due to slight differences in disease prevalence in the two patient populations and not the result of a diagnostic advantage of one strategy over the other. However, it is reassuring that a limited strategy does not result in lower disease detection, and thus the counterargument that a limited flow cytometry strategy would “miss” disease is not valid. In cases where no ancillary findings or clinical history are available or limited, employing a limited flow cytometry strategy as described would lead to similar results in the community setting.

In support of the above, our proposed limited flow cytometry strategy was successfully validated by a retrospective application of the limited strategy to cases that were positive by flow cytometry using the multimarker strategy. In 92.2% of the cases positive using the multimarker strategy, the abnormal/clonal population would have been detected using the limited flow cytometry strategy. In seven of the remaining cases, minor clonal plasma cell populations detected using the multimarker strategy would have been apparent by the clinical history of a monoclonal paraprotein and the lack of a clonal B-cell population by the limited flow cytometry strategy. The history of amyloidosis in the remaining case would have prompted immunohistochemical evaluation; in the validation study, a clonal plasma cell population was confirmed by immunohistochemistry.

Second, in our study population, myelodysplasia or acute leukemia was present in only a small fraction of cases (Figure 2). In the nonacademic setting such as ours where the

incidence of myelodysplastic syndrome (MDS) and AML is low, a limited flow cytometry strategy can identify cases of MDS or AML with a significant blast population using CD45 vs SSC gating (Figure 3). And thus, additional markers can be added to the flow cytometry study, if necessary, when the limited panel displays an increased blast population. In our experience, however, the need for additional markers following an initially selected limited panel based on smear findings is a rare event.

It may well be that the limited flow cytometry strategy is not as effective in the academic setting, where detection of minimal residual disease following therapy is often the goal, but we invite such laboratories to conduct a similar study to determine if the broad application of a limited flow cytometry strategy is feasible. In our population undergoing bone marrow staging for lymphoma, it may be possible to further reduce the number of flow cytometry markers used if the phenotype of the lymphoma is known; we did not address this question in the current study. We also did not address separately the issue of follow-up bone marrow examinations in monitoring disease relapse or progression; doing so may also lead to a further reduction in the number of markers used for this patient subpopulation.

Additional factors to consider in determining the feasibility of implementing a limited flow cytometry strategy include case volume, qualifications of the reviewers, and timely access to the bone marrow aspirate smears. Our experience is that the limited flow cytometry strategy is easily employed in a busy community setting, since both authors are not limited to hematopathology consultation. A limited strategy may be more easily applied in the academic setting where a flow cytometer is on site and where service duties are limited to one specialty, thereby allowing streamlining of workflow. In our laboratory, timely access to the smear would not be a factor in most cases since, as discussed above, the need for

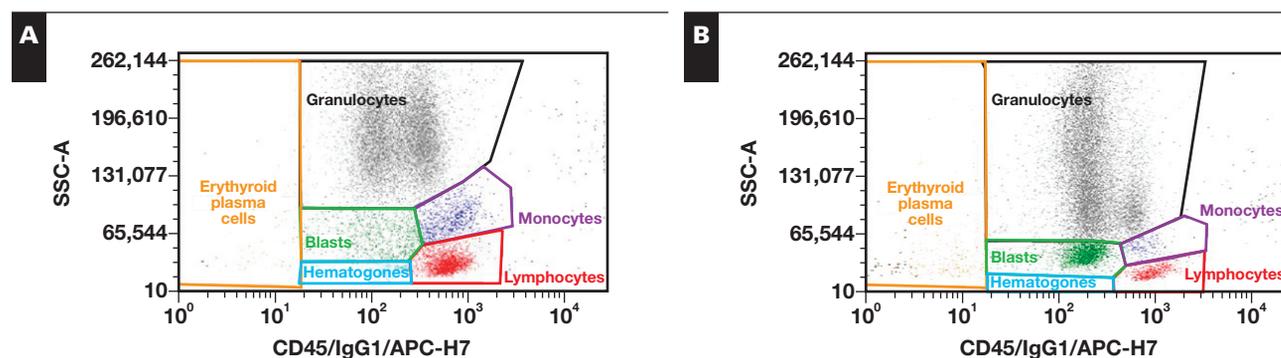


Figure 3 Flow cytometry CD45 vs side-scatter (SSC) plot of a normal bone marrow (A) and a case of myelodysplasia (B). Note the dual granulocyte population in the normal bone marrow. An increased, distinct blast population and shift in the granulocyte population in the case of myelodysplasia are evident compared with the normal study.

additional markers after a limited panel is performed is a rare event. Limited panels could be selected by nonpathologist personnel based on the clinical history provided, with additional markers added after the initial analysis by flow cytometry personnel based on a reflex testing protocol.

Our study did not evaluate limited flow cytometry panels in the evaluation of peripheral blood samples. However, we would expect that a similar strategy can be used on peripheral blood samples with similar results. This requires a similar study in a laboratory with a substantial volume of peripheral blood specimens submitted for flow cytometry, such as a commercial reference laboratory.

Key to the limited strategy is taking into account not only the bone marrow smear findings but also the patient's clinical history and clinical findings. Flow cytometric analysis is not indicated in evaluating staging bone marrows in cases of Hodgkin lymphoma or myeloproliferative disorders in the chronic phase.⁴ Also, in cases of plasma cell myeloma, it is apparent in most of our cases that the patient has a clonal population from serum or urine electrophoretic studies performed during clinical evaluation. Further evaluating for plasma cell clonality seems to be redundant and unnecessary when the neoplastic population is clearly evident by smear or biopsy morphology.

In our study, not performing flow cytometry did not lead to an increased use of immunohistochemical stains, which may be used instead to evaluate plasma cell dyscrasias. In addition, it is well known that flow cytometry underestimates plasma cell percentage compared with biopsy morphology because of hemodilution or loss of cells during specimen processing.¹² Despite this, even the use of a limited plasma cell panel, as previously described,¹³ would not significantly increase the mean number of markers used in the limited strategy because the percentage of plasma cell dyscrasia cases in our population is not disproportionately large.

Careful marker selection, rather than a broad panel approach to flow cytometric evaluation, will lead to significant cost savings if a limited flow cytometry strategy is adopted nationally. In our laboratory, cost savings for flow cytometry technical charges alone is estimated to be approximately \$125,000 annually. This strategy can be implemented without the need for additional staffing, additional processing, or increased workload, since smear preparation and review are already necessary for bone marrow examination.

With regard to cytogenetic testing on bone marrow samples, our study reveals that in cases with no abnormality detected by both flow cytometry and bone marrow morphology, as well as no history of CML, cytogenetics does not reveal any clonal abnormalities. And thus, in approximately 50% of cases (617 of 1,242 study cases), cytogenetic results, in retrospect, could have been reliably predicted to be negative by morphology and flow cytometric evaluation. This finding was regardless of the flow cytometry strategy used.

The frequency of fluorescence in situ hybridization (FISH) testing and other molecular testing in our laboratories was not high enough to determine if a limited flow cytometry strategy could predict negative FISH or other molecular testing results. Additional studies from laboratories with larger test volumes will be necessary to determine the value of a limited flow cytometry strategy in predicting negative results of these testing modalities.

In the absence of a history of CML, restricting cytogenetics to cases that display an abnormality by flow cytometry or morphology will result in significant cost savings. In our laboratory alone, cost savings using this strategy will result in approximately \$185,000 in savings annually.

In summary, using the limited flow cytometry strategy described in the evaluation of bone marrow samples, as well as the combined flow cytometry and morphologic findings to direct cytogenetic testing, will result in a savings of approximately \$310,000 annually for our patient population. Extrapolated nationwide, the projected cost savings, if these strategies are employed nationally, is approximately \$140 million.

Underlying the cost analysis is a set of assumptions that simplify our savings projections. These include no geographic variation in costs, a national population that is similar to our patient population, and the assumption that all laboratories follow a multimarker strategy. Hospital systems with very large patient populations may have lower flow cytometry marker and cytogenetics costs due to economies of scale, whereas smaller laboratories would have higher per unit costs; costs will also vary by geographic location. We did not take these factors into account in our analysis. Similarly, we assumed that all laboratories follow a multimarker strategy, and this not likely to be the case. Some laboratories, however, may use more flow cytometry markers than the multimarker strategy we describe, whereas other laboratories will use fewer markers. Thus, the mean number of markers used in the multimarker strategy described herein provides a reasonable estimate of the national average.

Our analysis also excludes the contribution of the pediatric population as well as more complex patient populations of tertiary care centers that may undergo more frequent bone marrow examination and require a larger flow cytometry panel or more frequent cytogenetic testing as the result of requirements for participation in clinical trials. Including these effects would likely increase our cost savings projection. Our national cost savings projection also does not include the cost savings that would be experienced by third-party payers, which would be significant, increasing the total cost savings to at least \$200 million annually. Thus, the national annual cost savings of using the limited flow cytometry strategy we describe is likely to result in a level of savings that is greater than our current estimate.

The cost benefit of a proactive stance by the evaluating pathologist in deciding which ancillary testing to perform on bone marrow samples is reflected in our findings, and its importance will be further emphasized as laboratories become more accountable for testing costs in the future. The implementation of the limited flow cytometry strategy we describe appears to be feasible in the community, academic, and reference laboratory settings.

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