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Cost Effectiveness of Cell Proliferation vs. Cytogenetics for Risk Stratification in Multiple Myeloma

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Introduction

Outcomes for multiple myeloma (MM) are heterogeneous. Choice of therapy requires risk stratification.[1] Staging is performed by the International Staging System (ISS).[2] Risk assessment also includes indicators of cancer behavior. Cytogenetics is routinely performed, yet is costly, time-consuming and often difficult to interpret.[3,4] Proliferation predicts MM outcomes and fulfills IMWG (International Myeloma Working Group) criteria for a myeloma-defining biomarker, but a practical assay is needed for clinical use.[5] Syndecan-1 (CD138) immunohistochemistry (IHC) on a trephine marrow biopsy is the most sensitive method of detecting myeloma cells.[6,7] Ki-67 is a cell proliferation nuclear protein shown to be associated with clinical outcomes in myeloma and many other tumor types.[8]

Design

We validated a multiplex IHC (mIHC) assay (formalin-fixed, paraffin-embedded marrow cores) in which coexpression of Ki67 (brown or black chromogen) is assessed in CD138+ (Syndecan 1; red chromogen) MM cells (Figure 1). 200 MM cells are manually counted as coexpressing Ki67 or not. The count is expressed as a percentage index (Syndecan + Ki + Index = "SynKii") (Biocare Medical). We performed a retrospective cohort study of 151 newly-diagnosed, treatment-naïve patients assessed by ISS, cytogenetics and SynKii (Table 1). Patients were divided by SynKii: Low (≤ 5%, n = 87), and High (>5%, n=64). Outcomes were compared. CMS (Centers for Medicare & Medicaid Services) data were queried for cost.

Table 1

Univariate and multivariate analysis of prognostic markers as predictors of overall survival.

Prognostic Variable	HR	95% CI	P	HR	95% CI	P
ISS > 1	2.59	1.47-4.58	0.001*	2.30	1.19-4.44	0.014*
High risk cytogenetics	2.28	1.32-3.95	0.003*	2.02	1.10-3.70	0.023*
Creatinine > 1.4 mg/dL	1.96	1.17-3.28	0.010*	1.27	0.68-2.36	0.454
Age > 65 years	1.88	1.15-3.05	0.012*	1.55	.911-2.63	0.106
SynKii > 5	1.64	1.01-2.66	0.046*	1.70	1.023-2.81	0.041*
Hemoglobin < 10 g/dL	1.45	0.868-2.42	0.156			
Marrow plasmacytosis > 15%	1.31	.564-3.04	0.529			
Calcium > 10 mg/dL	1.38	.784-2.42	0.265			
Presence of Extramedullary disease	1.42	.516-3.91	0.497			
CRP > 6 mg/L	1.12	.485-2.60	0.786			
LDH > 300 IU/dL	1.62	.802-3.28	0.18			

*Statistically significant

Figure 1

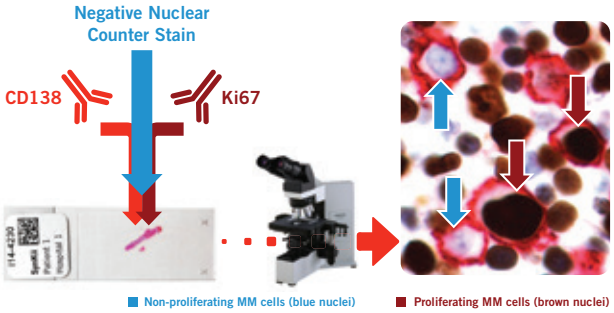


Figure 2

Overall Survival

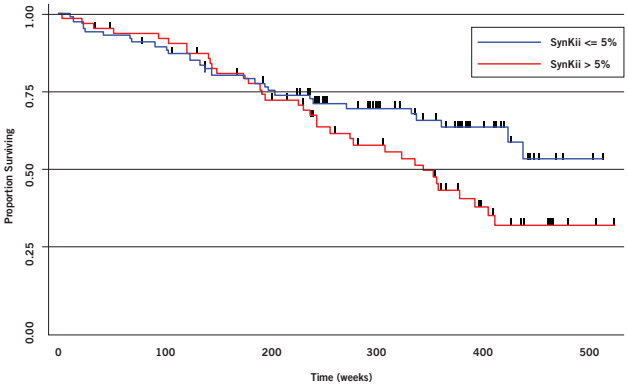


Figure 3

High risk patients categorized by overlap of SynKii, ISS and cytogenics

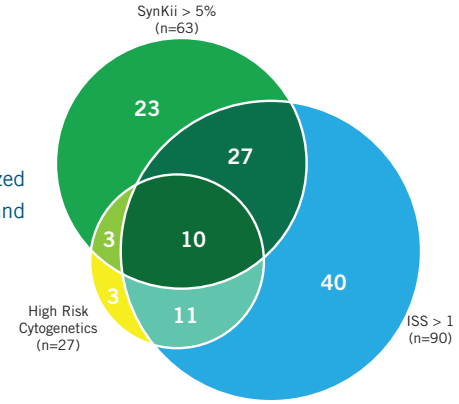
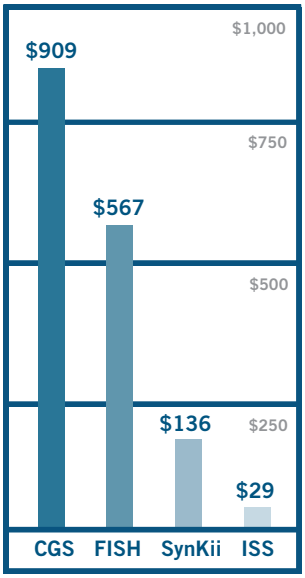


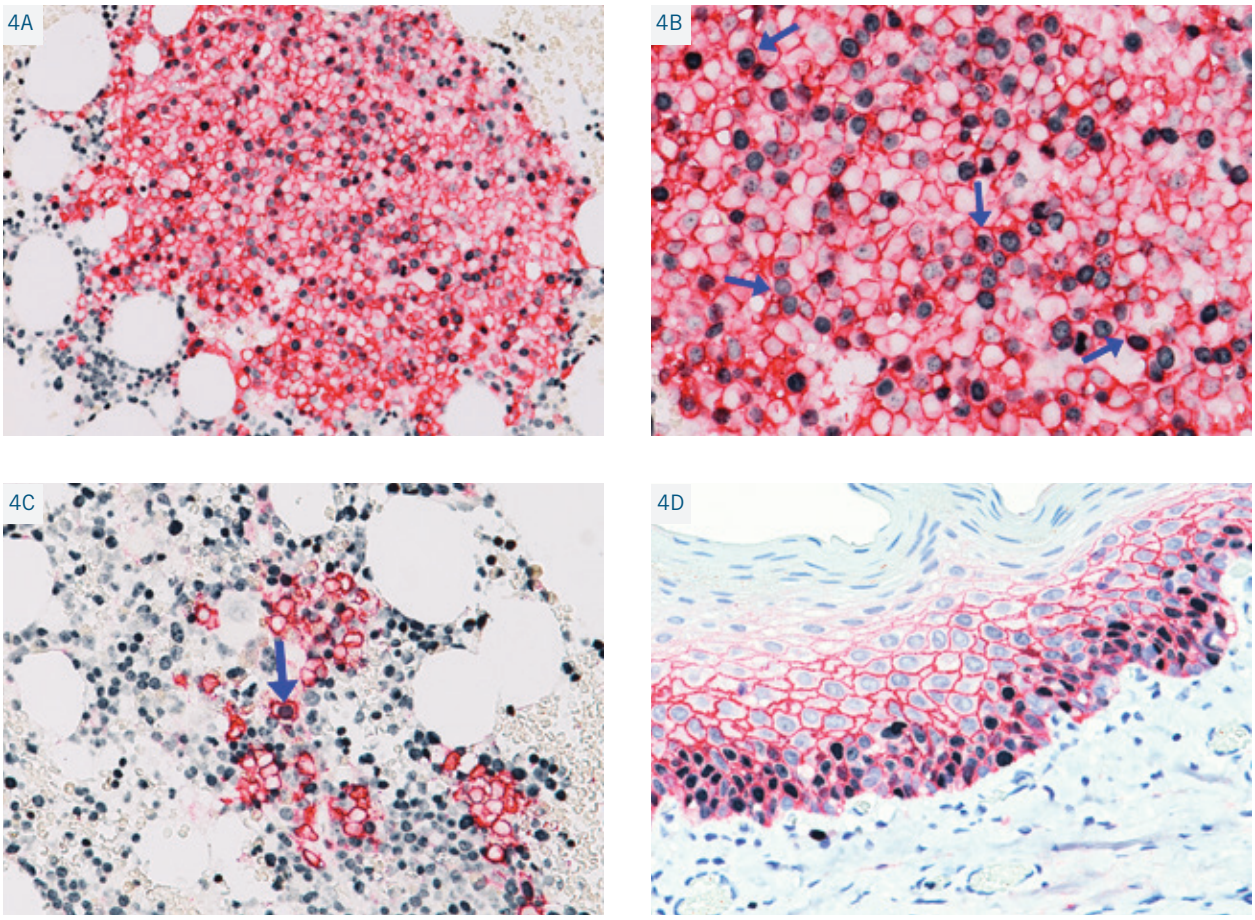
Table 2

CPT Code	Description	Medicare National Average Payment	*Average Units per Specimen	Total
88237	Cell culture for karyotype	$171.89	1	$171.89
88262	Chromosome banding (15-20 Cells)	$169.62	1	$169.62
88271	Single DNA probe	$29.14	9	$262.26
88275	FISH cell count (1 unit = 100-300 cells)	$54.65	5	$273.25
88291	FISH interpretation	$31.82	1	$31.82
88360	Synkii (manual count, multiplex IHC)	$135.87	1	$135.87
82040	Serum Albumin	$6.73	1	$6.73
82232	Serum beta-2 microglobulin	$22.00	1	$22.00
81479	***Gene array	N/A	1	$3,950.00
			**Average Total Karyotype and FISH	$908.84
			**Average Total FISH only	$567.33
			**Average Total SynKii	$135.87
			**Average Total ISS	$28.73



*The number of probes varies between 6 and 20 (most often 9). The number of cells counted increases with the number of probes (most often 5 units billed). **The averages provided reflect Medicare payments. Payments from private insurers vary. ***CMS does not publish fees for gene array testing. Signal Genetics list price is $3,950, but payments vary and are typically lower.

Figure 4



4A: Multiple Myeloma stained with CD138 (fast red) and Ki-67 (Black) 100X. 4B: Multiple Myeloma stained with CD138 (fast red) and Ki-67 (Black) 200X Arrows: (Notice Ki-67 nuclei are within the CD138 stained tumor cells). 4C: Multiple Myeloma stained with CD138 (fast red) and Ki-67 (Black) 200X Arrow: (Notice Ki-67 nuclei is within the CD138 stained tumor cells). Figure 4D Control Tissue: Stratified squamous epithelium in tonsil stained with CD138 (fast red) and Ki-67 (Black).

Results

The CD138 (red) + Ki-67 (brown or black) dual cocktail provides high contrast and easy assessment between proliferating MM cells and non-proliferating MM cells. Median overall survival was not reached vs. 78.9 months (P = 0.043) for low vs. high SynKii (Figure 2). In multivariable analysis, high-risk cytogenetics (HR 2.02, P = 0.023), ISS (HR 2.30, P = 0.014), and SynKii (HR 1.70, P = 0.041) had independent effects on overall survival (Table 1 and Figure 2). 60% (90/151) were ISS>1. There was overlap in high risk groups identified by ISS, cytogenetics and SynKii (Figure 3). However, SynKii identified 42% of patients (63/151) as high risk, compared to only 18% (27/151) identified by cytogenetics. three patients were identified as high risk by cytogenetics, but classified as standard risk by ISS and SynKii. Two of those three were among the longest survivors, suggesting they were misidentified by cytogenetics, but correctly classified by SynKii and ISS. CMS average costs are: ISS $28.73, SynKii, $135.87, and cytogenetics $908.84 (estimated average) (Table 2).

Discussion

There are numerous regimens for treatment of MM, ranging from oral drug/out patient to bone marrow transplantation. The choice in a given patient requires risk assessment. Staging is useful for this purpose, higher stage suggesting efficacy for more aggressive therapy. However, ideally, indicators of biologic behavior would be combined with staging. Cytogenetic analysis has traditionally been used. Most recognized genetic abnormalities function through increasing MM cell proliferation. Assessing proliferation directly is an established prognostic indicator, but the traditional *ex vivo* method is not feasible for modern clinical labs. Because myeloma cells are underrepresented in liquid aspirate specimens such as those used for flow cytometry,[6] we worked to devise a method that would instead use core biopsies, which the IMWG considers the gold standard for myeloma assessment.[5] In this background, we constructed an mIHC method using CD138 with a red chromogen, to identify MM cells. By manually counting 200 MM cells as either proliferating (brown nucleus, Ki67+) or not (blue counterstain), we found this method is clear and simple to use. To make the mIHC even simpler and more user friendly, we created a one-step SynKii kit for rapid use on automated immunostainers. Though the brown/red SynKii performed well, we found that the assay was improved by replacing the brown chromogen with a black chromogen and not using any counterstain (non-black, empty nuclei are negative)(Figure 4, A-D).

Conclusion

SynKii >5% and high risk cytogenetics independently predicted overall survival, but SynKii was more sensitive and accurate. Cytogenetics greatly increased the dollar cost but provided little additional relevant information. These data support the combination of ISS for staging and SynKii for myeloma cell proliferation, for cost effective risk assessment.

References

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