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ORIGINAL ARTICLE

MyD88 and TLR4 Expression in Epithelial Ovarian Cancer

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Abstract

Objective: To evaluate myeloid differentiation primary response gene 88 (MyD88) and Toll-like receptor 4 (TLR4) expression in relation to clinical features of epithelial ovarian cancer, histologic subtypes, and overall survival.

Patients and Methods: We conducted centralized immunohistochemical staining, semiquantitative scoring, and survival analysis in 5263 patients participating in the Ovarian Tumor Tissue Analysis consortium. Patients were diagnosed between January 1, 1978, and December 31, 2014, including 2865 high-grade serous ovarian carcinomas (HGSOCs), with more than 12,000 person-years of follow-up time. Tissue microarrays were stained for MyD88 and TLR4, and staining intensity was classified using a 2-tiered system for each marker (weak vs strong).

Results: Expression of MyD88 and TLR4 was similar in all histotypes except clear cell ovarian cancer, which showed reduced expression compared with other histotypes ($P<.001$ for both). In HGSOC, strong MyD88 expression was modestly associated with shortened overall survival (hazard ratio [HR], 1.13; 95% CI, 1.01-1.26; $P=.04$) but was also associated with advanced stage ($P<.001$). The expression of TLR4 was not associated with survival. In low-grade serous ovarian cancer (LGSOC), strong expression of both MyD88 and TLR4 was associated with favorable survival (HR [95% CI], 0.49 [0.29-0.84] and 0.44 [0.21-0.89], respectively; $P=.009$ and $P=.02$, respectively).

Conclusion: Results are consistent with an association between strong MyD88 staining and advanced stage and poorer survival in HGSOC and demonstrate correlation between strong MyD88 and TLR4 staining and improved survival in LGSOC, highlighting the biological differences between the 2 serous histotypes.

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Epithelial ovarian cancer (EOC) remains the fifth leading cause of cancer death among women in United States, resulting in more than 14,000 estimated deaths in 2017.[1] Less than 40% of patients with EOC are cured, because more than 70% of patients are diagnosed with advanced disease (stage III or IV).[2] The frustrating truth is that since platinum-based treatment was introduced more than 30 years ago, the overall survival rate of women with EOC has changed little[3]; most patients will relapse and die from their disease despite response to first-line surgery and chemotherapy.[4] Thus, novel therapeutic approaches for EOC are needed.[5]

The innate immune system recognizes the presence of bacterial pathogens through the expression of a family of membrane receptors known as Toll-like receptors (TLRs).[6] Although their expression is well established in immune cells, TLRs are also found in a myriad of human cancers, including EOC.[7] Among all the TLRs, Toll-like receptor 4 (TLR4) is perhaps the most extensively investigated.[8] Cumulating studies have suggested that coexpression of TLR4, along with myeloid differentiation primary response gene 88 (MyD88), a TLR signaling adapter protein, associates with poor prognosis in EOC by facilitating proliferation, survival, and chemoresistance of EOC cells through the activation of various proinflammatory cytokines and antiapoptotic proteins.[9-11] An additional report of atractylenolide-I, a novel TLR4-antagonizing agent, has been shown to sensitize EOC cells to paclitaxel by blocking the MyD88/TLR4 pathway.[12]

We previously analyzed immunohistochemical staining of MyD88 and TLR4 in a collection of approximately 450 EOC cases seen at Mayo Clinic; we found that the expression of both MyD88 and TLR4 was associated with poorer overall survival at $P< .05$.[13] Although adjustment of clinical covariates such as age, stage, histology, grade, and surgical debulking status attenuated these results, MyD88- and TLR4-associated risk estimates remained suggestive and indicated the need for larger analyses.[13]

Therefore, we here report analysis of an independent EOC study population of more than 10 times the previous sample size, allowing for a more detailed assessment of prognosis by histotype with consideration of combined expression and clinical subsets.

PATIENTS AND METHODS

Eligible cases with primary diagnosis of epithelial ovarian, peritoneal, or fallopian tube cancer were diagnosed between January 1, 1978, and December 31, 2014, and enrolled into 21 collaborative studies participating in the Ovarian Tumor Tissue Analysis consortium.[14-17] Mayo Clinic cases in the original report were

not included.[13] Histopathological classification for each case was provided by each contributing study, which was supplemented by additional pathologic review guided by WT1 and p53 IHC staining for most studies as described previously.[14] From among 7377 cases arrayed on tissue microarrays (TMAs), we excluded those found to be duplicated across studies (n=4), missing vital status at last follow-up (n=288), missing time to last follow-up (n=17), missing age at diagnosis (n=11), found to be nonepithelial (n=63), missing tumor behavior (n=2), or unable to be scored for both markers because of inadequate amount of arrayed tumor tissue (n=1729) (Supplemental Figure, available online at http://www.mayoclinicproceedings.org). Thus, up to 5263 cases were used for analysis, including 4694 with tumors of the 5 major invasive histotypes (high-grade serous ovarian carcinoma [HGSOC]; endometrioid ovarian carcinoma [ENOC]; clear cell ovarian carcinoma [CCOC]; mucinous ovarian carcinoma [MOC]; and low-grade serous ovarian carcinoma [LGSOC]). As described below, additional cases were excluded for one marker or the other. Supplemental Table 1 (available online at http://www.mayoclinicproceedings.org) presents characteristics of participating studies, including sample size, location, and data collection methods, and Supplemental Table 2 (available online at http://www.mayoclinicproceedings.org) presents clinical characteristics of analyzed cases.

Immunohistochemistry

Sections of TMAs were provided for centralized immunostaining at the Mayo Clinic, Rochester, Minnesota. For MyD88, the primary antibody was the rabbit polyclonal antibody NBP1-19785 (Novus Biologicals)[18] and the detection system was Polymer Refine Detection System (Leica). This system included the hydrogen peroxidase block, postprimary and polymer reagent 3,3′-diaminobenzidine (DAB), and hematoxylin. Immunostaining visualization was achieved by incubating slides for 10 minutes in DAB and DAB buffer (1:19 mixture) from the Bond Polymer Refine Detection System. To this point, slides were rinsed between steps with 1′ Bond Wash Buffer (Leica). Before and after DAB incubation, slides were rinsed in distilled water. For TLR4, the monoclonal antibody 3B6 (Novus Biologicals) was used as the primary detection antibody.[19] The detection system was the Envision Flex System (Dako); the reagents were the Flex Peroxidase-Blocking for 5 minutes, Flex/HRP for 20 minutes, and Flex DAB+ Chromogen/Substrate Buffer (1 drop of DAB per 1 mL of substrate buffer) for 5 minutes. Slides were counterstained for 5 minutes using Schmidt hematoxylin and molecular biology grade water (1:1 mixture), followed by several rinses in 1′ Bond wash buffer and distilled water; this is not the hematoxylin provided with the Refine kit. Once the immunochemistry processes were completed, slides were removed from the stainer and rinsed in tap water for 5 minutes. Slides were dehydrated in increasing concentrations of ethyl alcohol and cleared in 3 changes of xylene before permanent coverslipping in xylene-based medium.

Scoring

Figure 1 shows representative stains with each score for MyD88 and TLR4. For MyD88, multiple cores from a subset of 312 cases were evaluated blindly by 2 independent pathologists (M.K. and P.R.) using a 4-tiered system

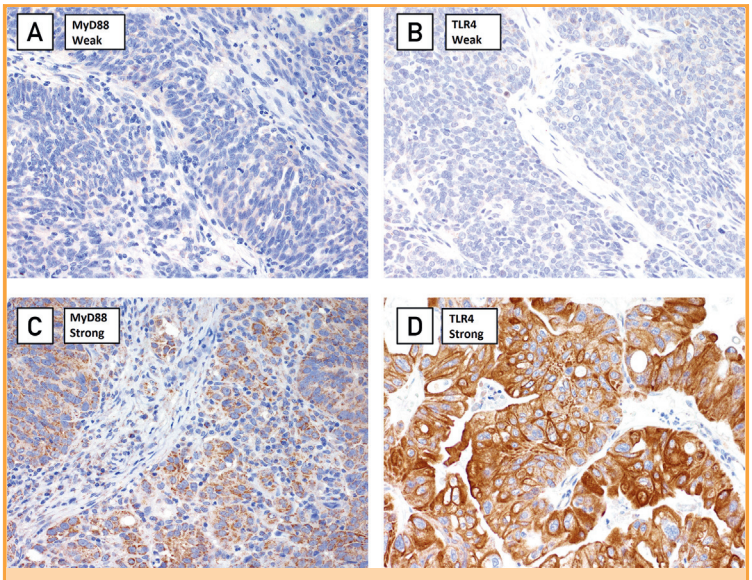

FIGURE 1. Representative immunohistochemical stains for MyD88 and TLR4 expression. A, High-grade serous carcinoma with weak MyD88 staining. B, High-grade serous carcinoma with weak TLR4 staining. C, High-grade serous carcinoma with strong MyD88 staining. D, High-grade serous carcinoma with strong TLR4 staining. MyD88 = myeloid differentiation primary response gene 88; TLR4 = Toll-like receptor 4.

(negative, weak, moderate, and strong), and a weighted kappa statistic was estimated. Then, differences in interpretation were discussed at a multiheaded microscope. Assessments of concordance in scores across these MyD88 cores indicated only modest levels of agreement (weighted κ, 0.55; 95% CI, 0.48-0.62); dichotomization of MyD88 expression into negative/weak and moderate/strong substantially increased agreement (κ, 0.81; 95% CI, 0.72-0.90). Because of this, all subsequent analyses of MyD88 and TLR4 expression used a dichotomous classification, henceforth referred to as weak (negative or weak expression) or strong (moderate or strong expression).

The cohort was subsequently split, with 51% of cases scored for MyD88 by M.K. and 49% by P.R. The TLR4 was evaluated by a single pathologist (M.K.). For cases with more than 1 scored core, the highest score was used in analysis. Because TMA cores with less than 25% epithelial tumor component were considered uninterpretable, a total of 137 cases were not scored for MyD88 (therefore, 5126 analyzed) and 676 cases were not scored for TLR4 (therefore, 4587 analyzed) (Supplemental Figure).

Analysis

Three sets of potential prognostic factors were evaluated: tumor expression of MyD88, of TLR4, and combinations of these 2. All statistical tests were 2-sided and uncorrected for multiple testing; all analyses were carried out using the SAS (SAS System, Inc) and R software (R Foundation for Statistical Computing) systems. Details on statistical analysis are provided in Supplemental Methods (available online at http://www.mayoclinicproceedings.org).

RESULTS

Clinical Characteristics of Study Cohort

The mean age at diagnosis for study participants was 58.0 years (Supplemental Table 2). Most of the cancers were advanced stage and high grade, and more than half of the patients were deceased as of last follow-up. Among the 5 main invasive EOC histotypes (N=4694), HGSOC was the most common (N=2865, 61%), followed by ENOC (N=670, 14%), CCOC (N=616, 13%), MOC (N=355, 7%), and LGSOC (N=188, 4%). Ten-year overall Kaplan-Meier survival estimates were 19% for HGSOC, 66% for ENOC, 50% for CCOC, 53% for MOC, and 30% for LGSOC.

Intracellular Distribution of Staining

The MyD88 staining distribution was mostly cytoplasmic with some membranous staining, consistent with the primarily cytoplasmic localization of the MyD88 adapter protein. Toll-like receptor 4 staining was distributed to both the cytoplasm and the plasma membrane. Toll-like receptor 4 is typically expressed both on the plasma membrane and in endosomes of myeloid leukocytes; localization of TLR4 expression on tumor cells has not previously been reported.

Distribution of Expression by Histotype

Distributions of MyD88 and TLR4 expression among the 5 most common invasive histotypes are presented in Table 1. In general, strong expression was more common than weak expression, with a notable exception: a minority of CCOCs (40%) showed strong TLR4 expression. Expression distributions for each marker were similar across HGSOCs, ENOCs, MOCs, and LGSOCs (68%-74% strong for MyD88; 71%-74% strong for TLR4). However, the expression in CCOCs was lower (only 59% strong for MyD88; only 40% strong for TLR4) compared with that of these histotypes combined (MyD88 $P<.001$; TLR, $P<.001$). Distributions among additional histopathological groups are provided in Supplemental Table 3 (available online at http://www.mayoclinicproceedings.org). Both MyD88 and TLR4 tended to be coexpressed, such that 67% of subjects either had low expression of both or high expression of both (χ^2 tests for independence $P<.001$, both overall and subset to the 5 most common invasive histotypes).

Clinical Correlates Among Women With HGSOC

Among women with HGSOC, associations of expression with clinical features are presented in Supplemental Table 4 (available online at http://www.mayoclinicproceedings.org). Expression was similar regardless of age at diagnosis ($P=.65$ for MyD88 and $P=.12$ for TLR4). High-grade serous ovarian carcinomas with strong MyD88 expression were more likely to be advanced stage than those with weak MyD88 expression (82% vs 75%, $P<.001$).

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TABLE 1. Associations of MyD88 and TLR4 Expression With Overall Survival Among Cases With the 5 Most Common Invasive Epithelial Ovarian Cancer Histotypes[a,b]

		MyD88				TLR4			
Histotype	Level	Subjects, n (%)	No. of events	HR (95% CI)	*P*	Subjects, n (%)	No. of events	HR (95% CI)	*P*
High-grade serous	Weak	712 (26)	450	Reference	.04	734 (29)	490	Reference	.35
	Strong	2064 (74)	1426	1.13 (1.01-1.26)		1788 (71)	1247	1.06 (0.94-1.18)	
Endometrioid	Weak	213 (32)	58	Reference	.82	169 (28)	48	Reference	.99
	Strong	447 (68)	109	0.96 (0.69-1.34)		443 (72)	105	1.00 (0.67-1.49)	
Clear cell	Weak	250 (41)	100	Reference	.96	335 (60)	142	Reference	.76
	Strong	358 (59)	158	1.01 (0.77-1.32)		226 (40)	93	1.04 (0.79-1.38)	
Mucinous	Weak	96 (28)	36	Reference	.64	79 (26)	40	Reference	.11
	Strong	249 (72)	96	1.12 (0.70-1.79)		224 (74)	71	1.54 (0.91-2.60)	
Low-grade serous	Weak	49 (27)	26	Reference	.009	42 (29)	21	Reference	.02
	Strong	133 (73)	64	0.49 (0.29-0.84)		103 (71)	54	0.44 (0.21-0.89)	

[a]HR = hazard ratio; MyD88 = myeloid differentiation primary response gene 88; TLR4 = Toll-like receptor 4.
[b]Adjusted for study, age (continuous), and stage (I/II, III/IV, unknown).

The expression of TLR4 did not differ by tumor stage (*P*=.75). Expression did not differ by extent of residual disease or known breast cancer susceptibility gene 1 (*BRCA1*) or breast cancer susceptibility gene 2 (*BRCA2*) pathogenic mutation.

Associations of Expression With Overall Survival by Histotype

Women with HGSOC and strong MyD88 expression had slightly poorer survival than did those with weak expression (unadjusted log-rank test *P*<.001; Figure 2). Results attenuated somewhat in age- and stage-adjusted Cox regression analyses but remained statistically significant (hazard ratio [HR], 1.13; 95% CI, 1.01-1.26; *P*=.04; Table 1). We found no evidence of a survival association with MyD88 expression for women with ENOC, CCOC, and MOC. Women with LGSOC and strong MyD88 expression had marginally better survival outcomes than did those with weak MyD88 expression (unadjusted log-rank test *P*=.14, Figure 2). This association strengthened after adjustment for study, age, and stage (Cox regression HR, 0.49; 95% CI, 0.29-0.84; *P*=.009; Table 1). Sensitivity analyses adjusting only for age and tumor stage demonstrated a similar protective effect of high MyD88 levels on survival (HR, 0.61; 95% CI, 0.39-0.97; *P*=.04).

For TLR4, even with nearly 12,000 years of follow-up for 2522 HGSOC cases (1737 deaths), we found no discernable evidence that survival differed by TLR4 expression. Kaplan-Meier curves overlapped considerably (Figure 3), and Cox regression analyses adjusting for age, stage, and study site were not statistically significant (HR, 1.06; 95% CI, 0.94-1.18; Table 1). There was some evidence for nonproportional hazards over time for women with HGSOC (*P*=.01). This can be seen in Figure 3, where the Kaplan-Meier curves cross in such a way that strong expression has a slight protective effect within the first 5 years of follow-up, then a slightly increased prognostic effect in years 5 through 10. However, in no instance is the difference between the 2 curves clinically meaningful. Moreover, time-stratified Cox regression analysis found no evidence of association with survival in either the first 5 years (*P*=.37) or the last 5 years of follow-up (*P*=.80).

We also did not observe any significant associations between TLR4 expression and overall survival for women with ENOC (Cox regression *P*=.99) or CCOC (*P*=.76) (Table 1). In univariate analyses, MOC tumors with strong TLR4 expression tended to fare better than those with weak expression (log-rank test *P*<.001; Figure 3). This association reversed direction in Cox regression analyses adjusting for study, age, and stage, such that MOC tumors with strong TLR4 expression fared more poorly than did those with weak expression, although the results failed to reach statistical significance (HR, 1.54; 95% CI, 0.91-2.60; *P*=.11; Table 1). Sensitivity analyses revealed that adjustment for study site had the most impact on this

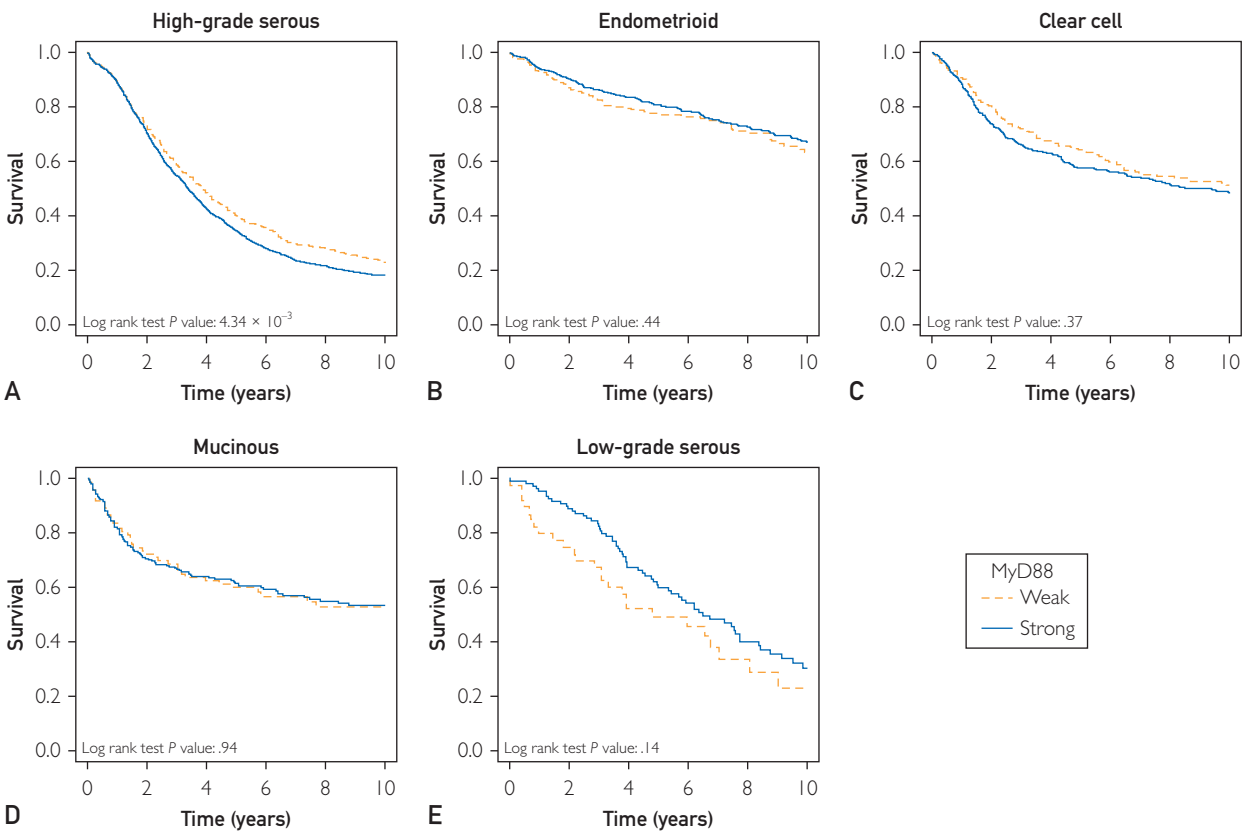

FIGURE 2. Kaplan-Meier overall survival plots by MyD88 expression for the 5 most common invasive ovarian cancer histotypes. A, High-grade serous ovarian cancer (N=2776); B, endometrioid ovarian cancer (N=660); C, clear cell ovarian cancer (N=608); D, mucinous ovarian cancer (N=345); and E, low-grade serous ovarian cancer (N=182). MyD88 = myeloid differentiation primary response gene 88.

change in direction of association: analyses adjusting for age and tumor stage only were essentially null (HR, 1.01; 95% CI, 0.67-1.54; *P*=.95). Kaplan-Meier curves displayed some evidence of a survival advantage for patients with LGSOC with strong TLR4 expression compared to those with weak expression. This effect was even more pronounced and reached statistical significance in adjusted Cox regression analyses (HR for strong expression compared with weak, 0.44; 95% CI, 0.21-0.89; *P*=.02; Table 1). As with MOC, analyses adjusting only for age and tumor stage were less striking (HR, 0.72; 95% CI, 0.43-1.20; *P*=.21).

Associations of Combined Expression With Overall Survival by Histotype

Because MyD88 and TLR4 coexpression has been shown to affect *in vitro* function,[9-11] we examined associations between survival and combination of expression (Figure 4 and Table 2). For women with HGSOC, those with strong MyD88 expression had poorer survival than those with weak MyD88 expression, regardless of TLR4 expression. As with the uncombined analyses in Table 1, we found no discernable patterns with survival and combinations of MyD88 and TLR4 expression in women with ENOC or CCOC (Cox regression *P*>.50 for each). Consistent with the associations observed in uncombined analyses, we saw little or no separation of survival curves for MyD88 expression, regardless of TLR4 expression, and we saw some association between high TLR4 expression and survival regardless of MyD88 expression for MOC tumors (*P*=.01; Figure 4). Again, consistent with uncombined analyses, associations

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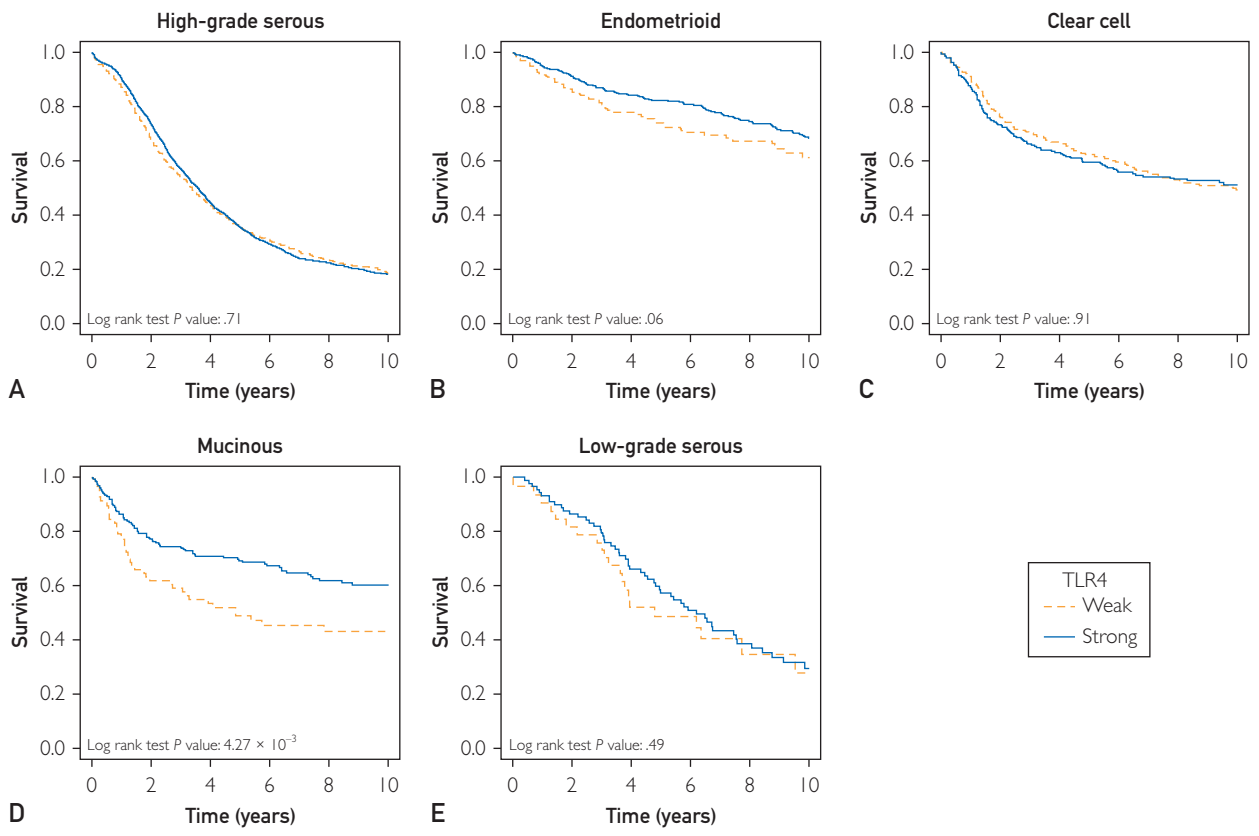

FIGURE 3. Kaplan-Meier overall survival plots by TLR4 expression for the 5 most common invasive ovarian cancer histotypes. A, High-grade serous ovarian cancer (N=2522); B, endometrioid ovarian cancer (N=612); C, clear cell ovarian cancer (N=561); D, mucinous ovarian cancer (N=303); and E, low-grade serous ovarian cancer (N=145). TLR4 = Toll-like receptor 4.

attenuated to nonsignificance after multivariate adjustment (Cox regression *P*=.52, Table 2). Compared with LGSOC tumors with weak expression of both markers, those with weak MyD88 and strong TLR4 expression (HR, 0.87; 95% CI, 0.28-2.71) and those with strong MyD88 and weak TLR4 expression (HR, 0.78; 95% CI, 0.28-2.21) had slightly longer survival. The LGSOC tumors with strong expression for both MyD88 and TLR4 had the longest survival times of the 4 expression groups (HR, 0.33; 95% CI, 0.13-0.85). We found no statistically significant survival interactions between MyD88 expression and TLR4 expression, either overall or by any of the histotypes in Table 1, indicating that effects of MyD88 expression on survival are not modified by expression of TLR4 and vice versa (interaction *P*>.05 for each).

Associations of Expression With Overall Survival in HGSOC Subsets

We evaluated the association between MyD88 and TLR4 expression and overall survival of HGSOCs by the extent of disease following surgery, *BRCA1* or *BRCA2* pathogenic mutation, and treatment (Table 3). For MyD88, the modest prognostic association remained; HRs were elevated in each group. For TLR4, there was a suggestion of effect modification by extent of residual disease (macroscopic disease: HR, 1.17; 95% CI, 0.98-1.39; *P*=.084; no macroscopic disease: HR, 0.76; 95% CI, 0.59-0.97; *P*=.03), but tests for interaction failed to reach statistical significance (*P*=.13). We also observed a potential prognostic association of TLR4 in *BRCA1* mutation carriers (HR, 2.69; 95% CI, 1.25-5.81; *P*=.01) and a potential protective effect in BRCA2 carriers (HR, 0.26; 95% CI, 0.08-0.84; *P*=.02), but tests for interaction

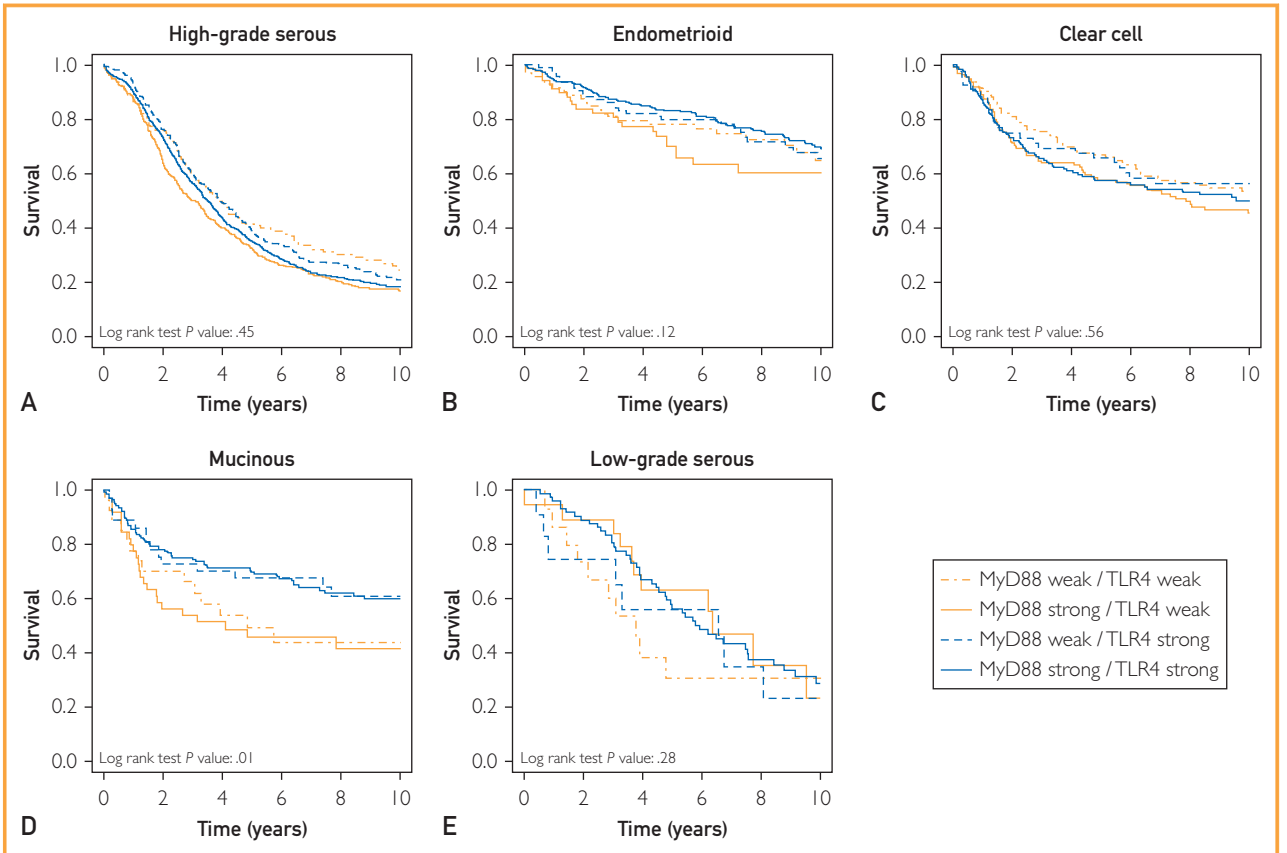

FIGURE 4. Kaplan-Meier overall survival plots by MyD88 and TLR4 expression for the 5 most common invasive ovarian cancer histotypes. A, High-grade serous ovarian cancer (N=2433); B, endometrioid ovarian cancer (N=602); C, clear cell ovarian cancer (N=293); D, mucinous ovarian cancer (N=553); and E, low-grade serous ovarian cancer (N=139). MyD88 = myeloid differentiation primary response gene 88; TLR4 = Toll-like receptor 4.

again failed to reach statistical significance (*P*=.08), perhaps due in part to small cell sizes within the mutation-positive groups. Combining MyD88 and TLR4 expression did not reveal additional patterns or interactions between the 2 expression values (Supplemental Table 5, available online at http://www.mayoclinicproceedings.org).

Additional Analyses

Because some previous ovarian cancer studies have combined LGSOC and HGSOC, we also analyzed invasive serous cases as a group, including those with missing grade. As expected, we found that combining the 2 groups together nullified the modestly higher expression-related associations observed in HGSOCs and the strikingly lower risks observed in LGSOCs (*P*=.21 for MyD88 and *P*=.49 for TLR4; see Supplemental Table 6, available online at http://www.mayoclinicproceedings.org). Combining LGSOCs with 127 serous borderline tumors (a potential precursor lesion) resulted in a similar protective association with strong MyD88 and TLR4 expression levels that we had observed in LGSOC-only analyses. No discernable patterns were observed between expression (including combined) and survival (including interactions) in other histological groups (Supplemental Tables 6 and 7, available online at http://www.mayoclinicproceedings.org), although results for many of these should be interpreted with caution because of low sample sizes.

Last, among the 5 major histotypes, time to disease progression was known for approximately half the cases. Progression-free survival results were similar to overall survival results

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TABLE 2. Associations of Combinations of MyD88 and TLR4 Expression With Overall Survival Among Cases With the 5 Most Common Invasive Epithelial Ovarian Cancer Histotypes[a,b]

Histotype	MyD88	TLR4	Subjects, n	No. of events	HR (95% CI)	*P*
High-grade serous	Weak	Weak	246	149	Reference	.10
		Strong	323	217	1.15 (0.92-1.42)	
	Strong	Weak	450	312	1.23 (1.01-1.51)	
		Strong	1414	991	1.25 (1.04-1.50)	
Endometrioid	Weak	Weak	85	22	Reference	.72
		Strong	103	27	1.18 (0.65-2.13)	
	Strong	Weak	75	23	1.40 (0.75-2.63)	
		Strong	339	78	1.08 (0.65-1.79)	
Clear cell	Weak	Weak	162	64	Reference	.82
		Strong	61	21	1.18 (0.70-1.99)	
	Strong	Weak	168	77	1.18 (0.82-1.69)	
		Strong	162	70	1.10 (0.76-1.60)	
Mucinous	Weak	Weak	28	14	Reference	.52
		Strong	43	13	1.73 (0.71-4.26)	
	Strong	Weak	44	23	1.05 (0.46-2.40)	
		Strong	178	57	1.43 (0.64-3.20)	
Low-grade serous	Weak	Weak	17	10	Reference	.02
		Strong	14	8	0.87 (0.28-2.71)	
	Strong	Weak	24	11	0.78 (0.28-2.21)	
		Strong	84	45	0.33 (0.13-0.85)	

[a]HR = hazard ratio; MyD88 = myeloid differentiation primary response gene 88; TLR4 = Toll-like receptor 4.
[b]Adjusted for study, age (continuous), and stage (I/II, III/IV, unknown). *P* values from unordered 3 degree-of-freedom test.

(Supplemental Tables 8 and 9, available online at http://www.mayoclinicproceedings.org). Note that the number of tests conducted is not small, no correction for multiple testing was done, and sample sizes are quite reduced in subset analyses.

DISCUSSION

Toll-like receptors, including TLR4, were originally described as myeloid leukocyte receptors for pathogen-associated molecular patterns, conserved structures present on subsets of pathogens but absent on normal host cells.[20] More recently, multiple endogenously expressed molecules (damage-associated molecular patterns) have been shown to stimulate myeloid cells via a TLR-dependent mechanism.[21] Specifically, TLR4 has been demonstrated to be stimulated by hsp60, hsp70, hsp90B1, heparan sulfate, fibrinogen, HMGB1, BD-2, and additional endogenous molecules.[21] Many of these damage-associated molecular patterns are present in the EOC microenvironment[13] and could therefore drive intracellular signaling through MyD88 and TLR4 in EOC cells expressing these proteins. The ligation of TLR4 triggers recruitment of the adapter protein MyD88 to the receptor complex. Upon recruitment, MyD88, in turn, recruits the kinases IL-1 receptor-associated kinase 1 and receptor-associated kinase 4. Once active, receptor-associated kinase 1 and receptor-associated kinase 4 recruit TNF receptor—associated factor 6, which activates IκB kinase. The activation of IκB kinase causes degradation of inhibitor of kappa B, promoting nuclear translocation and signaling by nuclear factor kappa B.[22] The signaling of nuclear factor kappa B generates proliferative and antiapoptotic signals in EOC cells.[23,24]

We and others have previously suggested that MyD88 expression is associated with poorer survival in EOC.[13,25] This association was confirmed in the present study only for patients with HGSOC, albeit with a relatively modest magnitude of effect after adjustment for age at diagnosis, disease stage, and study site. A likely explanation for the somewhat attenuated effect in the covariate-adjusted analysis is that MyD88 also is associated with advanced stage HGSOC. Thus, at least part of the impact of MyD88 on survival may be explained by its association with advanced disease. In sharp contrast with HGSOC,

TABLE 3. Associations of MyD88 and TLR4 Expression and Overall Survival Among HGSOC Cases by Extent of Residual Disease, by Pathogenic Mutation Status, and by First-Line Chemotherapy[a,b]

		MyD88				TLR4				
Clinical attribute	Level	N	No. of events	HR (95% CI)	*P*	Level	N	No. of events	HR (95% CI)	*P*
Extent of residual disease										
Macroscopic disease	Weak	274	202	Reference	.45	Weak	292	219	Reference	.08
	Strong	800	659	1.07 (0.90-1.27)		Strong	687	576	1.17 (0.98-1.39)	
No macroscopic disease	Weak	185	92	Reference	.39	Weak	207	115	Reference	.03
	Strong	561	278	1.12 (0.87-1.44)		Strong	476	238	0.76 (0.59-0.97)	
Pathogenic mutation status										
Tested negative	Weak	152	90	Reference	.02	Weak	147	99	Reference	.16
	Strong	485	347	1.33 (1.04-1.70)		Strong	444	321	1.19 (0.94-1.51)	
Pathogenic *BRCA1* mutation	Weak	23	11	Reference	.70	Weak	27	10	Reference	.01
	Strong	82	50	1.18 (0.51-2.72)		Strong	71	43	2.69 (1.25-5.81)	
Pathogenic *BRCA2* mutation	Weak	13	4	Reference	.69	Weak	11	6	Reference	.02
	Strong	35	15	1.30 (0.36-4.71)		Strong	31	12	0.26 (0.08-0.84)	
First-line chemotherapy treatment										
Standard treatment	Weak	59	36	Reference	.27	Weak	102	62	Reference	.77
	Strong	280	176	1.24 (0.85-1.82)		Strong	231	150	0.96 (0.68-1.37)	

[a]AUC = area under the concentration-time curve; *BRCA1* = breast cancer susceptibility gene 1; *BRCA2* = breast cancer susceptibility gene 2; HGSOC = high-grade serous ovarian cancer; HR, hazard ratio; MyD88 = myeloid differentiation primary response gene 88; TLR4 = Toll-like receptor 4.

[b]Adjusted for study, age (continuous), and stage (I/II, III/IV, unknown); mutation status reflects results of germline testing; standard treatment includes patients receiving ≥4 cycles of intravenous carboplatin AUC 5 or 6 and paclitaxel 135 mg/m^2 or 175 mg/m^2 every 3 wk and patients receiving ≥4 cycles of intravenous carboplatin and paclitaxel every 3 wk with dose presumed to be carboplatin AUC 5 or 6 and paclitaxel 135 mg/m^2 or 175 mg/m^2.

improved survival in LGSOC was associated with higher MyD88 expression. Furthermore, TLR4 expression also correlated positively with survival in LGSOC. Although the mechanism behind these divergent effects is not clear, the dichotomy of MyD88 and TLR4 associations between HGSOC and LGSOC adds further evidence that these 2 histotypes are biologically distinct. Understudied LGSOCs need additional investigation. Another of the understudied histotypes, MOC, showed a nonsignificant association between TLR4 expression and prognosis, although this result was more pronounced before accounting for the effects of study site; consideration of similarities with gastrointestinal tumors may help to unravel its biology.[26]

Although this study is the largest to examine the adaptor protein MyD88 and the pathogen-associated molecular pattern receptor TLR4, several significant limitations were unavoidable. First, the level of agreement between the 2 scorers for 4-scale MyD88 expression was modest, leading to the final analysis being performed on dichotomized data. A likely reason for the lower level of agreement with a 4-variable categorization is that the overall staining intensity of MyD88 was relatively modest. This change to a 2-variable categorization improved interscorer agreement but might limit the sensitivity of the analysis to detect subtle effects of MyD88 on survival. However, because this limitation would reduce sensitivity for detecting association between MyD88 staining intensity and survival, it would not be expected to contribute to false-discovery rate. We suggest that future studies consider other ways to measure MyD88 and TLR4 expression, perhaps by calculating the actual percent of cells positively stained.

CONCLUSION

The current study supports an association between increased MyD88 expression and poor survival in HGSOC and demonstrates that increased MyD88 expression is also associated with advanced stage HGSOC. In addition, the study suggests that both MyD88 expression and TLR4 expression are associated with favorable survival in LGSOC.

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SUPPLEMENTAL ONLINE MATERIAL

Supplemental material can be found online at http://www.mayoclinicproceedings.org. Supplemental material attached to journal articles has not been edited, and the authors take responsibility for the accuracy of all data.

Abbreviations and Acronyms: ***BRCA1*** = breast cancer susceptibility gene 1; ***BRCA2*** = breast cancer susceptibility gene 2; **CCOC** = clear cell ovarian carcinoma; **DAB** = 3,3′-diaminobenzidine; **ENOC** = endometrioid ovarian carcinoma; **EOC** = epithelial ovarian cancer; **HGSOC** = high-grade serous ovarian carcinoma; **HR** = hazard ratio; **LGSOC** = low-grade serous ovarian carcinoma; **MOC** = mucinous ovarian carcinoma; **MyD88** = myeloid differentiation primary response gene 88; **TMA** = tissue microarray; **TLR** = Toll-like receptor; **TLR4** = Toll-like receptor 4

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