Agent Orange Exposure, Cytogenetics, and Clinical Outcomes in Multiple Myeloma and MGUS Patients

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Abstract

Background While considerable research has examined transformation from monoclonal gammopathy of undetermined significance (MGUS) to multiple myeloma (MM) and the association of Agent Orange (AO) with MM, adverse cytogenetics are understudied.

Purpose To evaluate associations between AO and adverse cytogenetics or other poor prognostic factors such as abnormal light chain ratio in aged veterans with MM.

Materials and methods Vietnam-era Veterans diagnosed with MM or MGUS at a Veterans Health Administration site were identified. Chart review abstracted presence of 17p13, t(4;14), t(14;16), t(14;20), and Gain 1q by FISH (dubbed FISH-5) and q13/13q14 deletion, kappa/lambda ratio <.03 / >32 for MM (<.256 / >1.65 for MGUS), and bone marrow plasma cell percentage for MM.

Results Among 238 veterans, 70 had MM and 192 MGUS; 24 had both diagnoses (transformation). In MGUS, AO was not associated with transformation, although kappa/lambda ratio was. In MM, Hispanic veterans had more AO exposure. Survival was unassociated with AO or adverse cytogenetics, while Hispanic veterans experienced increased mortality.

Conclusion Our study explored cytogenetics in MM among surviving Vietnam Veterans with and without AO exposure. Although Hispanic veterans were more likely to have AO exposure, Hispanic ethnicity but not AO was associated with poorer survival.

Introduction

Multiple myeloma (MM) is a cancer of mature plasma cells in the bone marrow. MM accounts for 1–2% of cancers worldwide. Monoclonal gammopathy of unknown significance (MGUS) is typically the precursor to multiple myeloma, with approximately 1% of MGUS cases transforming to MM every year.[1]

Agent Orange (AO) exposure has been investigated as a risk factor for the transformation of MGUS to MM among Vietnam Veterans,[2-5] although a recent large Veterans Health Administration (VHA) database review found no association.[6] President John F Kennedy authorised Operation Ranch Hand in November 1961, which led to the US Air Force's herbicide program during the Vietnam War. Twenty million gallons of various chemicals were sprayed in Vietnam, eastern Laos and parts of Cambodia to defoliate rural land, depriving guerrillas of concealment in their support base and operations. AO was one of these chemicals; it was a mixed herbicide contaminated with traces of dioxin, a compound that has caused significant health problems among exposed individuals.[2]

Cytogenetics investigate chromosomal structure and function to discover potential treatment targets for specific diseases. Five major chromosomal mutations are associated with poor prognosis in patients with MM: 17p deletion, t(4;14), t(14;16), t(14;20) and gain of 1q. No study has evaluated the association between AO exposure and adverse cytogenetics or other poor prognostic factors, such as high bone marrow plasma cell percentage and abnormal light chain ratio in patients with MM. We hypothesised that AO exposure would be associated with adverse cytogenetics and that both AO and adverse cytogenetics would be associated with poor prognosis.

Methods

We reviewed the medical charts of Vietnam Veterans diagnosed with MGUS and/or MM in the Central Texas Veterans Health Care System to evaluate AO exposure, adverse cytogenetics, transformation from MM to MGUS, and overall survival. Inclusion criteria were active-duty military when AO was used (1961–1971) and MGUS or MM diagnosis at our site between October 1, 2009 and September 30, 2015 (prevalent cases FY2010–FY2015). Cases were excluded if there was illogical death data, missing data on age, race, ethnicity, or prior-year diagnostic data, or if MM/MGUS diagnosis could not be confirmed during chart review. Follow-up death data were available through April 1, 2020. Historical treatment data were unavailable. Regulatory review approval was obtained, and informed consent was waived.

MM was identified by ICD-9 diagnosis codes 203.00, 203.01, 203.02 and MGUS by code 273.1. Patients diagnosed with MM and also MGUS (at least three months apart) were considered 'transformation' patients. The presence of 17p13, t(4;14), t(14;16), t(14;20) or Gain 1q by fluorescence in situ hybridisation (FISH) defined high-risk cytogenetics (hereafter, 'FISH-5').[7–9] We also analysed 13q/13q14 deletion in addition to the above five aberrations.[7] A summary indicator of FISH-5 and/or 13q/13q14 deletion was also constructed ('FISH-6'). Poor prognosis per serum free kappa/lambda ratio was defined for MM patients as <0.03 or >32 K/L (high-risk K/L-ratio[10]), while kappa/lambda ratios <0.256 or >1.65 were considered potentially high-risk among MGUS patients.[11] Bone marrow plasma cell percentage being 60% or higher (hereafter, 'marrow-%') versus lower identified another biomarker of interest in MM.[12] Other measures included age at index date, gender, race, ethnicity, VHA Priority status (a value 1–8 summarising why the veteran qualified for VHA care such as military service-connected disability or very low income) and AO exposure per VHA enrolment and disability records. Assessment for AO exposure in the VHA is optional, so AO-exposed was contrasted with unexposed/undetermined.

Chi-square analysis or Student's t-test determined bivariate associations between AO and clinical indicators—transformation, FISH-5, FISH-6, q13/13q14 deletion, K/L ratio and marrow-% for patients with MM—as well as for age, Hispanic and Black. In a multivariable logistic regression, associations with transformation among MGUS patients were reported as odds ratios with their 95% confidence intervals (OR). Finally, a Cox proportional hazards model estimated associations of clinical and demographic variables with mortality among MM patients as hazard rate ratios with 95% confidence intervals (HR; criterion alpha 0.05) for AO status, age, race/ethnicity, transformation and cytogenetics.

Results

Among 238 veterans with MM or MGUS, four veterans were female, the average age was 65 years (SD: 5), 29% were Black and 11% were Hispanic. MGUS was diagnosed in 81%, MM 29%, and transformation 10%. Two-thirds of the cohort (64%, n=153) had documented AO exposure. AO status did not vary by Black race (65% of Black patients were AO-exposed vs 64% of non-Black patients; p=0.93) or Hispanic ethnicity (76% of Hispanics vs 63% of non-Hispanics; p=0.20) (see Table 1). FISH abnormalities were rare, with 6 MM patients having FISH-5 (32% of 19 with valid data), 8 having q13/13q14 deletion (42% of 19), and 11 having either or both (58% of 19). Nearly half (45%) the patients died by end of follow-up on April 1, 2020, more commonly those with MM (73% MM vs 34% MGUS-only). These two groups did not differ on age (MM with or without transformation 65.4 years (SD: 5.6) vs MGUS-only 64.9 years (SD: 4.5; t=0.61, df=107.5, p=0.54).

Among the 70 MM patients, Hispanic veterans were more likely to have had AO exposure (87.5% of Hispanic MM patients versus 46.8% of non-Hispanic MM patients, chi-square=4.7, df=1, p=0.03). AO was not associated with Black race or age, nor with FISH-5 nor with q13/13q14 deletion or their composite measure, FISH-6. Similarly, the transformation was unassociated with AO among MM patients (Table 2). Among MGUS patients (n=192), the K/L ratio was associated with transformation (OR=6.2, 95% CI 2.2–17.6; Table 3). In the multivariable survival model for MM patients (N=70), Hispanic ethnicity was associated with higher mortality (HR=10.5; 95% CI 1.1–97.2), while AO was unrelated (Table 4).

Discussion

Among Vietnam-era Veterans, ethnicity was a mortality risk factor, while AO was unrelated to survival. Our chart review sample did not reveal associations of cytogenetic abnormalities with survival, although low power may explain this result. Furthermore, the preliminary data on q13/13q14 deletion are intriguing although, again, clearly sparse and beg further examination. In our study, with 5–10 years of follow-up after cohort entry, these aging veterans experienced a 50% mortality rate. It may be useful to continue examining these cytogenetics in larger samples or samples with other toxic exposures, for example, among veterans exposed to burn pits.[13, 14]

Table 1. Characteristics of 192 MGUS patients and 70 MM patients, including 24 with both MGUS and MM (N=238)

	MGUS†	MM†
Characteristic	**N (%) or Mean ± SD**	**N (%) or Mean ± SD**
Age (54-77)	64.79 ± 4.79	65.38 ± 5.62
Charlson Comorbidity Index minus cancer (0-8)	1.61 ± 1.66	1.27 ± 1.33
Survival in years (0.17–11.5)	6.97 ± 2.78	4.95 ± 3.07
Survival status		
Alive as of April 1, 2020	119 (62.0%)	19 (27.1%)
Died during study period	73 (38.0%)	51 (72.9%)
Gender		
Female	4 (2.1%)	1 (1.4%)
Male	188 (97.9%)	69 (98.6%)
Race/Ethnicity		
White	107 (55.7%)	44 (62.9%)
Black	61 (31.8%)	15 (21.4%)
Hispanic	19 (9.9%)	8 (11.4%)
Other races	5 (2.6%)	3 (4.3%)
Marital status		
Married	123 (64.1%)	46 (65.7%)
Divorced/separated	53 (27.6%)	21 (30.0%)
Single	6 (3.1%)	1 (1.4%)
Widow(ed)	10 (5.2%)	2 (2.9%)
VHA Priority Group		
Group 1 50–100% disability	113 (58.9%)	45 (64.3%)
Groups 2/3/4 disability	41 (21.4%)	11 (15.7%)
Group 5 low income	38 (19.8%)	14 (20.0%)
Agent Orange exposure	131 (68.2%)	36 (51.4%)
No Agent Orange exposure	43 (22.4%)	11 (15.7%)
Agent Orange exposure undetermined	18 (9.4%)	23 (32.9%)
Transformation (overlap of MGUS + MM)	20 (10.4%)	24 (34.3%)
High-risk cytogenetics per FISH among MM patients (missing: 51)		
FISH-5 †	-	6 (31.6%)
FISH-6 (FISH-5 or q13/13q14 deletion) †	-	11 (57.9%)
q13/13q14 deletion	-	8 (42.1%)
Bone marrow plasma cell among MM patients (missing: 19)		
0-59%	-	39 (76.5%)
60-100%	-	12 (23.5%)
MGUS: K/L Ratio† is <.256 or >1.65 vs Other Status	86 (44.8%)	-
MM: K/L ratio is <0.03 or >32 vs Other status	-	26 (37.1%)

† **FISH=fluorescence in situ hybridisation; K/L=serum free kappa/lambda; MGUS=monoclonal gammopathy of undetermined significance; MM=multiple myeloma**

Table 2. Associations with Agent Orange exposure in patients with multiple myeloma (n=70)

Characteristic *	AO-exposed† (n=36)	Non-AO-exposed (n=11)	Undetermined AO (n=23)
Transformation (MGUS+MM) †	14 (39%)	5 (45%)	5 (22%)
High-risk K/L ratio†	15 (42%)	5 (45%)	6 (26%)
High-risk cytogenetics: FISH-5† (missing: 51)	2 (18%)	1 (33%)	3 (60%)
High-risk cytogenetics: q13del† (missing: 51)	5 (45%)	1 (33%)	2 (33%)
High-risk cytogenetics: FISH-5 or q13del (missing: 51)	5 (45%)	2 (67%)	4 (80%)
Bone marrow plasma cell 60%+ (missing: 19)	7 (25%)	1 (9%)	4 (33%)

*** No significant associations per chi-square analysis**

† AO=Agent Orange; FISH=fluorescence in situ hybridisation; K/L=serum free kappa/lambda; MGUS=monoclonal gammopathy of undetermined significance; MM=multiple myeloma; q13del=q13/q1314 deletion

Table 3. Logistic regression on transformation among 192 Patients with MGUS

Characteristic	Odds ratio	95% Confidence interval	p-value
Demographic correlates			
Agent Orange exposure	0.62	0.26–1.49	0.281
Age	0.96	0.87–1.05	0.342
Hispanic	0.82	0.17–3.96	0.801
Black	0.99	0.38–2.55	0.982
Adding K/L ratio			
Agent Orange exposure	0.53	0.21–1.35	0.184
Age	0.96	0.88–1.05	0.376
Hispanic	0.69	0.14–3.49	0.650
Black	0.81	0.30–2.20	0.678
K/L ratio – MGUS * †	6.15	2.15–17.60	0.001

*** 95% confidence interval excludes 1.0**

† K/L=serum free kappa/lambda; MGUS=monoclonal gammopathy of undetermined significance

Table 4. Predictors of mortality among 70 veterans with multiple myeloma

Characteristic	Hazard rate ratio	95% Confidence interval	p-value
Agent Orange exposure	2.23	0.60–8.19	0.23
Age in years	1.06	0.95–1.19	0.29
Hispanic *	11.02	1.18–103.00	0.04
Black	0.43	0.08–2.29	0.32
Transformation	1.46	0.32–6.74	0.63
FISH-5†	4.91	0.34–70.27	0.24
FISH-q13del†	1.18	0.19–7.17	0.86

*** 95% Confidence interval excludes 1.0**

† FISH=fluorescence in situ hybridisation; q13del=q13/13q14 deletion

While the FISH-5 cytogenetic mutations appear to confer an overall poor prognosis, studies have identified chromosome 13 mutations (in particular, chromosome 13 monosomy and 13q deletion) as factors in haematologic malignancies. Yet, there is no consensus on their value in clinical application. Binder et al. suggest that the presence of chromosome 13 monosomy denotes a poorer prognosis with shorter overall survival, whereas 13q deletion confers a favourable prognosis with relatively longer survival.[7] This is the only study delineating monosomy 13 and 13q deletion effects and associations in MM. Previous studies report a poor prognosis with any chromosome 13 abnormalities.[15, 16] Furthermore, Zojer et al. describe the clinical application of 13q14 deletion in MM and note a poor prognostic relationship, with an overall increase in proliferation of myeloma cells with this mutation.[17] Still, other studies describe an overall poor prognosis associated with 13q deletion more in association with typically concomitant high-risk genetic mutations (e.g., the FISH-5) in patients with MM.[16–18] In our study, 4% of veterans had a 13q deletion mutation. Given the rarity of this abnormality in our sample, its role as a causative factor cannot be supported or disputed. Given our centre's limited FISH and cytogenetic data, we could not distinguish between patients with monosomy 13 and 13q deletion abnormalities. These mutations merit further evaluation in patients diagnosed with MM.

The association of high-risk kappa/lambda ratio among MGUS patients with transformation to MM confirms the value of this marker of poor prognosis. Currently, we use the kappa/lambda ratio in conjunction with other markers to stratify patient risk of transformation to MM. A deeper study of the identified risk levels (<0.256 or >1.65), rate of change in kappa/lambda ratios, or alternative cut-points and their correlations with novel markers could enhance the clinical value of the kappa/lambda ratio as personalised medicine expands.

Previously, Black patients were shown to have twice the incidence of MM with a poorer prognosis, attributed to racial disparities and socioeconomic status; this is especially important to recognise given new data suggesting Black individuals have a lower prevalence of FISH-5 mutations, specifically 17p deletion and have a higher prevalence of the protective t(11;14) mutation.[19, 20] Conversely, our data showed that Hispanic individuals had more AO exposure and overall higher mortality. Kaur et al. suggest that Hispanics have a higher incidence of MM compared to non-Hispanic whites and have a higher comparative incidence of renal dysfunction but with similar survival to their White and Black counterparts given equal access to therapy.[19, 21] Buradagunta et al. propose that higher mortality in Hispanic individuals may be driven by comorbidity associated with socioeconomic factors.[22]

Limitations

Our chart review was limited to a single site with a small sample size with scant data on marrow-%. Nevertheless, it provides preliminary evidence of how cytogenetics could be used to inform clinical status in patients with complex disease profiles.

Conclusion

Continued study is merited to fully recognise what predisposes MM development following AO or other cytotoxic exposure and whether novel military exposures are engendering this and other cancers of the lymphatic system. Scarcely half a million of the three million American military personnel who served in Vietnam from 1954–1975 are alive today. Their experiences have fuelled many medical inquiries and innovations, leading to better care in the field and rapid evacuation for Gulf War and Global War on Terror Veterans.[23–25] We suspect that continued exploration of cytogenetic and epigenetic factors in disease aetiology will continue to advance our ability to care for today's veterans and those who served 50 years ago.

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