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Early life mebendazole exposure increases the risk of adult-onset ulcerative colitis: a population-based cohort study

Authors: Manasi Agrawal, MD, MS^{1,2}, Kristine H. Allin, MD, PhD^{1,3}, Aske T. Iversen, MSc¹, Saurabh Mehandru, MD^{2,4}, Jean-Frederic Colombel², MD, Tine Jess, MD, DMSc^{1,3}

Authors' institutions:

1. Center for Molecular Prediction of Inflammatory Bowel Disease (PREDICT), Aalborg University, Department of Clinical Medicine, Copenhagen, Denmark
2. The Dr. Henry D. Janowitz Division of Gastroenterology, Icahn School of Medicine at Mount Sinai, New York, NY
3. Department of Gastroenterology & Hepatology, Aalborg University Hospital, Aalborg, Denmark
4. Precision Institute of Immunology, Icahn School of Medicine at Mount Sinai, New York, NY

Corresponding author: Manasi Agrawal, MD, MS

Contact information:

The Henry D. Janowitz Division of Gastroenterology
Icahn School of Medicine at Mount Sinai
1 Gustave L. Levy Place
New York NY 10029
Email: manasi.agrawal@mountsinai.org

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Abstract

Background

According to the hygiene hypothesis, exposure to parasites may protect against inflammatory bowel disease (IBD). Our aim was to examine the risk of IBD with childhood exposure to mebendazole, a broad-spectrum antihelminthic agent.

Methods

We conducted a population-based cohort study using prospectively collected historical data of all individuals born in Denmark between 1995 and 2018. We identified mebendazole exposure at age <18 years, as well as during early life (<5 years of age). We performed adjusted Cox proportional hazards regression analysis to determine the risk of IBD, ulcerative colitis (UC) and Crohn's disease (CD) with mebendazole exposure after adjusting for potential confounders.

Results

Of 1,520,290 individuals in the cohort, 615,794 had childhood or adolescence mebendazole exposure. 1,555 and 1,499 individuals were subsequently diagnosed with pediatric- and adult-onset IBD, respectively. On multivariable analysis, mebendazole exposure at <18 years of age did not impact pediatric- or adult-onset IBD risk (aHR 0.97, 95% CI 0.87, 1.07 and 1.08, 95% CI 0.97, 1.19, respectively). On limiting mebendazole exposure to age <5 years, while there was no association with pediatric-onset IBD (aHR 0.98, 95% CI 0.87, 1.11), adult-onset IBD risk was increased (aHR 1.17, 95% CI 1.04, 1.31). This increase in risk was driven by UC (aHR 1.32, 95% CI 1.12, 1.55), but not CD (1.03, 95% CI 0.87, 1.22).

Conclusion

Early life mebendazole exposure is associated with increase in the risk of adult-onset UC. These findings suggest the importance of early life exposures in shaping the risk of IBD later in life.

Study highlights

WHAT IS KNOWN

- The incidence of inflammatory bowel diseases is rising in developing countries.
- Exposure to helminth infestations influences immune tolerance.
- Exposures during the early life period modulate immune function, microbiome composition and the risk of chronic diseases.

WHAT IS NEW HERE

- Mebendazole exposure at less than five years of age, but not later in childhood, is associated with an increased risk of adult-onset UC.
- Mebendazole exposure is not associated with pediatric-onset UC or with CD.

Background

Inflammatory bowel disease (IBD), a chronic immune mediated disease of the intestinal tract, includes ulcerative colitis (UC) and Crohn's disease (CD), which are characterized by colonic mucosal and gastrointestinal transmural inflammation, respectively.¹ While the etiology of IBD is multifactorial and not well-understood, environmental exposures that modulate mucosal immune function and intestinal microbiome may attenuate (e.g. *Helicobacter pylori* infection) or enhance (e.g. antibiotics) the risk of IBD.^{2,3} The "hygiene hypothesis", which suggests that childhood exposure to infections and infestations may promote immune tolerance and thereby protect against immune-mediated and allergic diseases, has received attention in this regard.⁴

Helminth larvae have been detected in fossilized animal feces originating from the Lower and Middle Pleistocene eras, even predating the evolution of humans as a species.⁵ Humans have therefore co-evolved not only with microorganisms but also with metazoan parasites.⁶ Since parasites evolve at a rate that is slower than viral or microbial agents and establish into small human communities, they perhaps represent a "stable threat" to their human hosts. Therefore, in comparison to viruses and bacteria, parasites are considered to have imparted a stronger genetic signature on the human immune system especially on some of the innate immune pathways.⁷ Stated differently, parasitic infections resemble successful 'xenotransplants' that reset the thresholds of host immune reactivity in order to escape expulsion from the host. In doing so, parasitic infestations may also dampen responses to allergens as well as exogenous and auto-antigens, and exert a protective role against inflammatory diseases such as IBD.⁸ An important variable in this regard is the timing of infestation. The early life period, considered to extend from the prenatal phase to five years of age, is a window of susceptibility for immune development, and exposures during this period can confer long-lasting effects on the offspring and modulate risk for chronic, immune-mediated diseases after a long latent period.^{3,9}

Highly effective, broad-spectrum antihelminthic therapies, such as mebendazole, are prescribed to symptomatic patients, and can serve as surrogates for helminth infestations in epidemiological studies in countries with adequate healthcare access, and therefore would be expected to have a protective effect against IBD. Mebendazole is a benzimidazole derivative, which when administered orally, blocks cellular tubulin formation leading to degenerative changes in the intestinal tissues of helminths residing in the host gastrointestinal tract.¹⁰ It is also important to consider the timing of helminth eradication; if during early life, this could

potentially eliminate or decrease the “tolerogenic effect” of the infestation and thereby have the opposite effect.

Therefore, the aim of this study was to examine the impact of mebendazole therapy on the future risk of IBD, as well as the impact of therapy during the early life period on this risk. We leveraged historical, prospectively collected data from cross-linked Danish registers of all individuals who lived in Denmark to conduct a nationwide cohort study.

Methods

Study population

We used the Danish Civil Registration System (CRS) to identify all individuals who were born in Denmark between January 1, 1995, and December 31, 2018 and included them as the study population.

Exposure: mebendazole prescription

The CRS number cross-links the CRS to the Danish National Prescription Register, which records data on all prescribed medications since January 1, 1995. In Denmark, the standard of care is prescription of two doses of mebendazole to the infected individual for the treatment of any helminth infestation, which we defined as primary exposure, as well as to all household members for presumed co-infection, which we defined as secondary exposure.¹¹ Using data from the National Prescription Register, we identified all individuals who were prescribed mebendazole and the age at the first ever prescription. We let exposure status change from unexposed to exposed at the first prescription.

Outcome: Crohn’s disease or ulcerative colitis

The CRS number also cross-links the CRS to the Danish National Patient Register which records hospitalization data since 1977, and ambulatory and emergency room encounters since 1995. Using the International Classification of Diseases Eighth and Tenth Revisions (ICD-8, ICD-10) codes, we identified CD and UC diagnoses in the register (CD: ICD-8 code category 5630 and ICD-10 code category K50; UC: ICD-8 code category 56319, 56904 and ICD-10 code category K51), defined as two or more outpatient or inpatient encounters or at least one inpatient encounter ≥ 7 days with a diagnosis code of CD or UC. The positive predictive value of two ICD-10 codes in the National Patient Register for CD and UC diagnosis is estimated to be

0.94 (95% CI 0.9, 0.97) and 0.89 (95% CI 0.85, 0.92), respectively while the positive predictive value of one ICD-10 code was slightly lower [0.91 (0.86-0.94) and 0.85 (0.81-0.89), respectively].¹² For individuals whose medical records included both CD and UC diagnosis codes, the diagnosis at the second encounter was considered. The time of diagnosis was considered to be the second in or outpatient contact or 7 days into the first contact, whichever occurred first. IBD onset was considered to be pediatric or adult when it was diagnosed at age <16 years or ≥16 years, respectively.¹³

Covariates

We included the following covariates: calendar period (divided into three equal categories 1995-2002, 2003-2010, 2011-2018), number of siblings and half-siblings (0,1, 2 and ≥3), antibiotic prescription (yes, no), sex (female, male), socio-economic status in quartiles, degree of urbanization (low, medium and high) and parental IBD status (yes, no). Individual records in the CRS are linked with those of first-degree relatives through the CRS number, using which, we identified parents, siblings, half-siblings and offspring. Data on age and sex were also obtained from the CRS, while those on antibiotic prescription were obtained from the National Prescription Register. The area-level socioeconomic status and degree of urbanization are official summary statistics linked with the addresses of all individuals in the CRS.^{14, 15} Information on parental IBD status was obtained from the National Patient Register.

Statistical analysis

We followed all individuals in the cohort from birth until CD or UC diagnosis, death, emigration or December 31, 2018, whichever occurred first. We used unadjusted and multi-factor adjusted Cox proportional hazards regression models to estimate hazard ratios (HRs) of IBD combined, and CD or UC separately, censoring at opposite IBD subtype.

In our primary analysis, we modeled the hazards of the outcome IBD with any mebendazole exposure, primary or secondary, at age <18 years, vs no mebendazole exposure at age <18 years, adjusting for all covariates with a p value <0.10 on univariable analyses. These included female sex (p=0.02), antibiotic exposure (p<0.0001), calendar period (<0.001), degree of urbanization (p=0.05) and parental IBD status (p <0.0001). All variables but the last could be a potential confounder in the association between mebendazole exposure and IBD and were therefore included in multivariable models. While parental IBD status is a well-known risk factor for IBD in the offspring, it would not impact mebendazole prescription. Therefore, we did not

include it in the primary models, but did include it in additional analyses. For each analysis, we differentiated between pediatric versus older onset IBD, based on age at onset <16 or ≥16 years of age.¹³

In order to delineate the impact of early life mebendazole exposure, we analyzed the hazards of IBD with any exposure to mebendazole at age <5 years, vs no exposure at age <5 years. We then conducted separate analyses to determine the hazards of CD and UC as outcomes by any exposure, and early life exposure to mebendazole, as above. As the definition of “early life” is variable, we also conducted a sensitivity analysis to determine the estimates of each outcome with exposure to mebendazole at age <3 years. We conducted an additional sensitivity analysis to determine the impact of primary mebendazole exposure vs no exposure. We determined the absolute risk of IBD with exposure to mebendazole.

Mebendazole exposure, calendar period, number of siblings and half-siblings, antibiotic prescription and parental IBD status were treated as time-varying variables, while sex, socioeconomic status and degree of urbanization were fixed at birth. Proportional hazards assumption was analyzed graphically, and was satisfied when we modeled the hazards of pediatric and adult-onset IBD separately.

Statistical analyses were conducted using the SAS statistical software version 9.4 (SAS Institute, Cary, North Carolina).

Patient and public involvement

Patients or the public were not involved in the design, conduct, reporting, or dissemination plans of this research.

Ethics

After approval by the Danish Data Protection Agency, all analyses were conducted on a secure server provided by the Danish Health Data Authority. Registry-data based research is exempt from ethical approval in Denmark.

Results

Of 1,520,290 individuals in the cohort, 615,794 had any exposure, primary (250,542) or secondary, to mebendazole at age <18 years; 348,934 were exposed to mebendazole at age <5 years. The mean age (standard deviation, SD) of first mebendazole exposure was 5.1 (3.6) years. In the overall cohort, 740,061 (48.7%) individuals were of female sex. The other baseline demographic and clinical characteristics of the cohort are described in Table 1. 1,555 and 1,499 individuals were subsequently diagnosed with pediatric- and adult-onset IBD, respectively. The number of individuals with UC and CD outcomes, person-years of follow up, and median age at outcome are reported in the supplementary table.

IBD risk with exposure to mebendazole at <18 years of age

On univariable analysis, exposure to mebendazole at age <18 years, compared to no exposure during this age period, was not associated with pediatric- or adult-onset IBD (HR 0.98, 95% CI 0.88, 1.09 and HR 1.09, 95% CI 0.99, 1.21, respectively). On multivariable analysis, after adjusting for sex, parental IBD status, antibiotic exposure, calendar period of mebendazole exposure and degree of urbanization, the risk of IBD, both pediatric- and adult-onset, remained unchanged among individuals who received mebendazole at <18 years of age (aHR 0.97, 95% CI 0.87, 1.07 and aHR 1.08, 95% CI 0.97, 1.19, respectively).

IBD risk with exposure to mebendazole at <5 years of age

On limiting univariable analysis to mebendazole exposure at age <5 years, compared to no exposure during this time period, it was not associated with pediatric-onset IBD (HR 0.98, 95% CI 0.87, 1.11) but it was associated with adult-onset IBD (HR 1.18, 95% CI 1.05, 1.32). On multivariable analysis, while there was no increase in hazards of pediatric-onset IBD (aHR 0.98, 95% CI 0.87, 1.11), the hazards of adult-onset IBD was increased with mebendazole exposure at <5 years of age (aHR 1.17, 95% CI 1.04, 1.31). The estimates remained unchanged after adjusting additionally for parental IBD status (Table 2). The cumulative incidence curve of IBD is reported in Figure 1.

IBD subtype

On analyzing the hazards of the outcomes UC and CD with mebendazole exposure at age <5 years, censoring at the opposite disease type, we observed that the increased adult-onset IBD risk was driven by UC (aHR 1.32, 95% CI 1.12, 1.56), but not CD (1.04, 95% CI 0.88, 1.23).

Pediatric-onset UC and CD were not associated with mebendazole exposure at <5 years of age (aHR 1.02, 95% CI 0.85, 1.22 and 0.96, 95% CI 0.81, 1.13, respectively). The estimates remained unchanged when parental IBD status was added to the models. Cumulative incidence curves of IBD are reported in Figures 2a and b.

Absolute risk

We observed 9.1 and 7.7 cases of adult-onset IBD for every 10,000 person years of (adult) follow-up in individuals with and without early life mebendazole exposure, respectively. There were 1.4 excess cases of adult-onset IBD per 10,000 person years with early life mebendazole exposure.

Sensitivity analyses

We conducted additional sensitivity analyses, restricting mebendazole exposure to <3 years, compared with no exposure during this time period. We found that the risk of adult-onset IBD remained unchanged compared to estimates with mebendazole exposure at <5 years (aHR 1.16, 95% CI 1.00, 1.34).

When limiting the univariable analysis to primary exposure versus no exposure to mebendazole at age <18 years, the HR for pediatric and adult-onset IBD was 1.05 (95% CI 0.92, 1.21) and 1.06 (95% CI 0.93, 1.22). Similarly, in adjusted models, the risk of IBD, both pediatric- and adult-onset, was not increased (aHR 1.04, 95% CI 0.91, 1.20 and 1.04, 95% CI 0.91, 1.19, respectively).

We calculated the E-value to estimate the minimum strength of association an unmeasured confounder would be required to have with both the exposure (mebendazole exposure at <5 years of age) and the outcome (adult-onset UC) to explain the observed association between the two.¹⁶ The E-value for the point estimate was 1.97 (1.49 for the lower confidence limit). To put this estimate into context, we determined the corresponding estimate for the association of parental IBD status with the exposure and with the outcome. The HR for mebendazole exposure at <5 years of age was 1.09 for children of parents with IBD vs children of parents without IBD, and the HR for adult-onset UC was 3.15 for children of parents with IBD vs children of parents without IBD. An unmeasured confounder of this magnitude would not be able to explain the observed association nor render it statistical insignificant.

Discussion

Our study, conducted in a large, nationwide cohort of well-characterized individuals from Denmark, reports an association between early-life (<5 years of age) exposure to mebendazole, a broad-spectrum antihelminthic drug, and the risk of adult-onset IBD after adjusting for relevant confounding variables. In contrast, this risk was not observed if the exposure occurred later in the childhood or adolescence. This increase in IBD risk was driven by increase in UC, but not CD, risk. To our knowledge, this is the first study to implicate antihelminthic therapy during the early life period in future UC risk.

In a previous population-based cohort study from Denmark between 1995 and 2008, Bager et al found no association between antiparasitic therapy at <14 years of age and the risk of IBD up to 14 years of age.¹⁷ These findings are consistent with the null association between childhood or adolescence exposure to mebendazole and IBD in our analysis. Our finding that mebendazole exposure increases UC risk only when it occurs at <5 years of age is novel and further underscores the relevance of the early life period towards immune modulation and risk of future IBD.³ The Developmental Origins of Health and Disease hypothesis suggests that the early life period is a critical window of susceptibility, and upon exposure to various environmental agents, the developing fetus and young child develop adaptive immune responses, which carry long term health consequences.⁹ The early life period, and exposures during this time, are also critical to the composition and stability of the gut microbiome, which is implicated in host-microbe interactions.^{4, 18} In a systematic review with meta-analysis, early life was an important period of susceptibility for IBD development later in life. Tobacco smoke, infections and antibiotics were associated positively, and breastfeeding was associated negatively with IBD.³ A recent study demonstrated that early, but not late, restitution of the microbiome reduced colitis risk in mice models with antibiotic-induced dysbiosis.¹⁹

We also report some important null results. Early life exposure to mebendazole was associated with an increase in adult-onset, but not pediatric-onset, UC. This could be due to two reasons. First, pediatric-onset IBD versus adult-onset IBD have differential risk factors and pathogenesis, with a larger contribution of genetic risk in the former group.²⁰ Second, there is likely to be a long lag period from exposure to environmental insults, progressive alteration in immune function, preclinical phase, until disease diagnosis. Increasing data indicate that early life exposures can lead to disease outcomes much later in life, the study of which is referred to as

“life course epidemiology”.^{3, 21} Early life exposure to mebendazole was associated with an increased risk of UC, but not CD. We postulate that altered mucosal immune function due to helminths may modulate the risk of UC, a mucosal disease, more so than that of CD, which involves transmural inflammation. Differences in type 2 immunity, including those in innate lymphoid cells type 2, type-2-cell-mediated immunity and regulatory T cells may also be at play.¹ Further mechanistic data will be informative. *Trichuris suis* ova as treatment for UC and CD have been studied; but so far the data do not clearly demonstrate efficacy.²²

Our findings provide a unique insight underpinning the rise of IBD incidence, and especially UC incidence, in developing countries in parallel with implementation of helminth eradication programs and decrease in soil-transmitted helminth prevalence.^{23, 24} Based on these data, we cannot distinguish whether the increased risk of UC with mebendazole exposure is mediated indirectly via eradication of helminthic infestation implying a protective role of helminthic infestations against UC, or directly through a harmful effect of the drug. Prior data have reported an association between parasitic infestation and IBD.²⁵ There are several lines of evidence on intestinal immune modulation by soil-transmitted helminths in support of the former argument. Helminth infection is strongly associated with the induction of type-2-cell-mediated immunity (T2CM), with the possibility that T2CM may have evolved as a response to helminth parasites.²⁶ T2CM, especially those mediated by the innate immune system, are ascribed key homeostatic roles.²⁷ T2CM is also associated with goblet cell hyperplasia and induction of epithelial cytokines such as IL-25, IL-33 and thymic stromal lymphopoietin.²⁸⁻³² In addition to innate T2CM, the adaptive responses of T2CM include the induction of regulatory T cells.³³ Altogether, the net result of the T2CM induced by helminth infestation is to protect barrier tissues from damage.²⁸ Intestinal helminths infestation has also been associated with higher microbiome diversity; and eradication may alter the microbiome diversity and composition, with reduction of Clostridiales and increase in Bacteroidetes.^{4, 34} Finally, the direct harmful effect of mebendazole may include damage to the epithelial barrier. Mebendazole is a microtubule inhibitor that achieves high concentration in the intestinal lumen.¹⁰ Theoretically, mebendazole may impede host microtubule structures, especially in the rapidly dividing intestinal epithelial cells, causing cytotoxicity to the intestinal epithelium and impairing the barrier function of the intestines. Further studies are needed to uncouple the indirect and direct effects of mebendazole.

The strengths of our study include the prospective, nationwide and unselected cohort of individuals over a 24 year period. There was no loss to follow up, and we adjusted for potential

confounding variables. We conducted sensitivity analysis using different definitions of the early life period, as well as primary versus secondary mebendazole exposure. Our study also has some limitations. There is a risk of misclassification of exposure, as well as outcome. In Denmark, all medication prescriptions are recorded consistently in the National Patient Register, and the likelihood of misclassification of exposure is low. As mebendazole is the only antihelminthic drug prescribed in Denmark, the impact of other drugs in the class could not be assessed. Furthermore, misclassification, if any, is likely to be non-differential, and thereby bias the results towards the null. The definition of the outcome variable using two diagnoses codes has been previously validated against the pathology register, although this study used ICD-8 codes, and against a population-based inception cohort using ICD-10 codes.^{12, 35} In addition to using two separate records, we included IBD patients with one contact lasting seven days or more to account for patients having a long in- or outpatient contact. Since the patients with one long hospital contact accounted for 9.97% of included patients, we suggest that the sensitivity and specificity of our definition would be closer to that of the definition using two codes rather than that using one. Given that these are historical data and an observational study, there is the risk of unmeasured and residual confounding. However, the E-value estimate suggests that this is unlikely.

In summary, in this population-based cohort study, we report that early life, but not later in childhood or adolescence, exposure to mebendazole is associated with an increase in the risk of adult-onset UC. These results are consistent with the importance of early-life exposures in shaping the risk of IBD later in life. Further data are needed to replicate and further substantiate these findings, understand differences between UC and CD risk factors and pathways, and for re-evaluation of risks and benefits of large-scale helminth eradication programs.

Table 1: Characteristics of the cohort by exposure

	All		Exposed to mebendazole before age 5 years		Exposed to mebendazole between age 5 and 18 years		Not exposed to mebendazole before age 18	
	N	%	N	%	N	%	N	%
All	1,520,290	100.0	348,934	100.0	266,860	100.0	904,496	100.0
Sex								
Female	740,061	48.7	179,527	51.5	133,158	49.9	427,376	47.3
Male	780,229	51.3	169,407	48.5	133,702	50.1	477,120	52.7
Area socio economic index								
Q1	372,515	24.5	92,315	26.5	71,878	26.9	208,322	23.0
Q2	394,122	25.9	90,967	26.1	61,166	22.9	241,989	26.8
Q3	384,737	25.3	84,083	24.1	57,483	21.5	243,171	26.9
Q4	368,916	24.3	81,569	23.4	76,333	28.6	211,014	23.3
Degree of urbanization								
High	587,931	38.7	127,719	36.6	103,710	38.9	356,502	39.4
Medium	463,771	30.5	106,949	30.7	80,799	30.3	276,023	30.5
Low	468,588	30.8	114,266	32.7	82,351	30.9	271,971	30.1
Antibiotic use								
No	260,318	17.1	39,241	11.2	12,930	4.8	208,147	23.0
Yes	1,259,972	82.9	309,693	88.8	253,930	95.2	696,349	77.0
Number of siblings (maximum)								
0	185,791	12.2	14,687	4.2	11,357	4.3	159,747	17.7
1	673,569	44.3	141,920	40.7	113,506	42.5	418,143	46.2
2	427,338	28.1	120,098	34.4	88,325	33.1	218,915	24.2
≥3	233,592	15.4	72,229	20.7	53,672	20.1	107,691	11.9

Table 2: Crude and adjusted estimates for outcome with exposure to mebendazole in early life (<5 years of age)

	IBD onset	HR (95% CI)
Outcome: IBD		
Unadjusted model	Pediatric	0.98 (0.87, 1.11)
	Adult	1.18 (1.05, 1.32)
Adjusted model	Pediatric	0.98 (0.87, 1.11)
	Adult	1.17 (1.04, 1.31)
Adjusted model (also adjusted for parental IBD)	Pediatric	0.98 (0.86, 1.10)
	Adult	1.17 (1.04, 1.31)
Outcome: UC		
Unadjusted model	Pediatric	1.01 (0.84, 1.21)
	Adult	1.33 (1.13, 1.56)
Adjusted model	Pediatric	1.02 (0.85, 1.22)
	Adult	1.32 (1.12, 1.56)
Adjusted model (also adjusted for parental IBD)	Pediatric	1.01 (0.84, 1.21)
	Adult	1.32 (1.12, 1.55)
Outcome: CD		
Unadjusted model	Pediatric	0.96 (0.81, 1.13)
	Adult	1.05 (0.88, 1.23)
Adjusted model	Pediatric	0.96 (0.81, 1.13)
	Adult	1.04 (0.88, 1.23)
Adjusted model (also adjusted for parental IBD)	Pediatric	0.95 (0.80, 1.13)
	Adult	1.03 (0.87, 1.22)

Figure 1: Cumulative incidence curves for IBD

Figure 2: Cumulative incidence curves for (a) UC and (b) CD

Supplementary material -<http://links.lww.com/AJG/C632>

Visual abstract -<http://links.lww.com/AJG/C633>

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