Clinical Pharmacogenetics Implementation Consortium (CPIC) Guidelines for Rasburicase Therapy in the Context of *G6PD* Deficiency Genotype

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Glucose-6-phosphate dehydrogenase (G6PD) deficiency is associated with development of acute hemolytic anemia (AHA) induced by a number of drugs. We provide guidance as to which *G6PD* genotypes are associated with G6PD deficiency in males and females. Rasburicase is contraindicated in G6PD-deficient patients due to the risk of AHA and possibly methemoglobinemia. Unless preemptive genotyping has established a positive diagnosis of G6PD deficiency, quantitative enzyme assay remains the mainstay of screening prior to rasburicase use. The purpose of this article is to help interpret the results of clinical *G6PD* genotype tests so that they can guide the use of rasburicase. Detailed guidelines on other aspects of the use of rasburicase, including analyses of cost-effectiveness, are beyond the scope of this document. Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines are published and updated periodically on https://www.pharmgkb.org/page/cpic to reflect new developments in the field.

FOCUSED LITERATURE REVIEW

A systematic literature review focused on *G6PD*, glucose-6-phosphate dehydrogenase (G6PD) deficiency, and rasburicase use was conducted (see **Supplementary Material** online (Focused Literature Review)); reviews[1–8] were used to summarize background information. This is the first Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline to focus on *G6PD* and the first to focus on a pharmacogene on the X chromosome. Although G6PD deficiency increases susceptibility to oxidative stress induced by many other agents besides rasburicase, for many of them, the recommended prescribing modifications are unclear, whereas for rasburicase prescribing must clearly be modified based on G6PD status, as many countries' drug labels contraindicate rasburicase use in those with G6PD deficiency. Future CPIC guidelines will address other drugs affected by G6PD.

GENE: *G6PD*

Background

G6PD is the enzyme that converts glucose-6-phosphate into 6-phosphogluconolactone, the first step of the pentose phosphate pathway (PharmGKB; https://www.pharmgkb.org/pathway/PA165971634).[5] At the same time, G6PD produces reduced nicotinamide adenine dinucleotide phosphate (NADPH) from nicotinamide adenine dinucleotide phosphate. G6PD is ubiquitously expressed, and it is particularly important in erythrocytes because in these cells G6PD, along with 6-phosphogluconate dehydrogenase (another enzyme in the pentose phosphate pathway), is the only available source of NADPH.[2,3,9] NADPH is required to protect erythrocytes from oxidative stress.[8–10] Oxidative stress can be imposed by various substances, including oxygen radicals and hydrogen peroxide, that may be generated physiologically or may result from exposure to exogenous agents such as therapeutic drugs.[8,9,11] G6PD-deficient erythrocytes have a much reduced capacity for NADPH production; therefore, they are defective in handling oxidative stress and are thus more susceptible to drug-induced lysis (including from rasburicase), which can manifest clinically as hemolytic anemia.[2,3,8,9] Furthermore, oxidation of hemoglobin iron results in the formation of methemoglobin, a form of hemoglobin that cannot carry oxygen or carbon dioxide. Methemoglobinemia is defined as a condition in which the methemoglobin level is >1% in circulating blood and manifests clinically with a discoloration of the skin that simulates cyanosis and in severe cases may lead to arrhythmias, seizures, and death.[10–12]

Currently there are more than 180 reported genetic variants of *G6PD* (**Supplementary Table S1** online; see **Supplementary Material** online (*G6PD* Genetic Variant Nomenclature and

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WHO Class)).[13–15] Most of the genetic variants in *G6PD* are missense variants resulting in single amino acid substitutions; a few are in-frame deletions of one or more amino acids.[14] The lack of large deletions and of frameshift variants appears consistent with the finding that a complete absence of G6PD enzyme activity is fatal *in utero*, as has been shown in *G6PD* knockout mice.[16] Most of the genetic variants that result in low G6PD enzyme activity affect enzyme stability, with the most severe variants (usually in-frame deletions) causing alterations predominantly at or near the dimer interface of the G6PD protein in exon 10, which affects dimer formation and substrate binding.[9,14]

Historically, genetic variants of *G6PD* have been divided into five classes,[3] with class I being the most severely dysfunctional and class V having the highest enzyme activity. The classification was based on two criteria: (i) the level of G6PD activity in red blood cells and (ii) clinical presentation of individuals bearing those variants (**Supplementary Table S2** online). Given that *G6PD* is on the X chromosome, the classifications were primarily based on assessments in males; with only one copy of the gene, their erythrocyte enzyme activity reflects the only allele they carry (see **Supplementary Material** online (*G6PD* Genetic Variant Nomenclature and WHO Class and *G6PD* heterozygotes) and **Supplementary Tables S1 and S2** online).[3,15,17] *G6PD* variants are defined as class I when they are associated with chronic nonspherocytic hemolytic anemia (CNSHA): They are found in patients who have hemolysis even in the absence of any challenge and usually have a G6PD activity less than 10% of normal in red blood cells.[3,15,18] Thus, patients with class I variants have a recognizable life-long clinical syndrome; in addition, class I variants are very rare (many of them have been reported only once). Class II variants are those with G6PD activity less than 10% but without CNSHA. Class III variants are those with G6PD activity between 10 and 60% of normal. Variants in class II and III are asymptomatic most of the time (by definition there is no CNSHA), but they are clinically important because they entail the risk of drug-induced acute hemolytic anemia (AHA). Of the millions of people who are G6PD deficient, nearly all carry at least one class II or a class III variant. Because, by definition, class II variants have a mean G6PD enzyme activity lower than that of class III variants, it stands to reason that AHA may be more severe with the former than with the latter. However, this does not mean that class III variants can be regarded as "mild"; for example, in trials of dapsone in children with G6PD A– (class III), 98% of G6PD-deficient males developed AHA, 11% of whom required blood transfusion.[19] Thus, a sharp division between class II and III variants may no longer be clinically useful.[18] Class IV comprises variants with normal activity: the large majority have G6PD B, but about 20% of those of African ancestry have G6PD A.[18] Class V is reserved for variants with higher-than-normal activity; however, none have been reported since the report of G6PD Hektoen.[18]

For the purpose of this guideline, the categories of G6PD phenotypes considered (**Table 1**) are G6PD deficient with CNSHA, G6PD deficient, G6PD normal, and G6PD variable.[18] Thus, nearly all individuals classified as "G6PD deficient" are those with WHO class II or class III variants; they are usually asymptomatic (without CNSHA) but are still at risk of AHA, favism, and neonatal jaundice.[18] Individuals classified in the "variable" category consist of females who carry one nondeficient (class

Table 1 Assignment of likely G6PD phenotypes based on genotype/diplotype

Likely phenotype	Definition	Genotypes	WHO class for *G6PD* variants[a]	Example of diplotypes[b]
Normal	Very mild or no enzyme deficiency (>60% of normal enzyme levels)	A male carrying a nondeficient (class IV) allele	IV	B, Sao Boria
		A female carrying two nondeficient (class IV) alleles	IV/IV	B/B, B/Sao Boria
Deficient	<10–60% of normal enzyme activity	A male carrying a deficient (class II–III) allele	II, III	A–, Orissa, Kalyan-Kerala, Mediterranean, Canton, Chatham
		A female carrying two deficient (class II–III variants) alleles	II/II, II/III, III/III	A–/A–, A–/Orissa, Orissa/Kalyan-Kerala, Mediterranean/Mediterranean, Chatham/Mediterranean, Canton/Viangchan
Deficient with CNSHA	Severe enzyme deficiency (<10% activity) and associated with CNHSA	A male carrying a class I allele	I	Bangkok, Villeurbanne
		A female carrying two deficient (class I variants) alleles	I/I	Bangkok/Bangkok, Bangkok/Villeurbanne
Variable[c]	Normal or deficient enzyme activity[c]	A female carrying one nondeficient (class IV) and one deficient (class I–III variants) allele	IV/I, IV/II, IV/III	B/A–, B/Mediterranean, B/Bangkok

CNSHA, chronic nonspherocytic hemolytic anemia; WHO, World Health Organization.

[a]WHO classifications from ref. 14, other details from ref. 17. Class I variants are extremely rare; the distinction between class II and III variants is not clear, and the "class V" very high activity variant has been reported in only a single case.[17,18] Therefore, almost all patients will carry class II, III, or IV alleles. It should be noted that the class of a variant may have been assigned only by the clinical manifestations of a patient in which the variant was subsequently identified. [b]Due to the large number of *G6PD* variants, many other diplotypes may be possible besides those given as examples here; see **Supplementary Table S1** online for a more comprehensive list of variant alleles with their assigned WHO class. [c]Due to X-linked mosaicism, females heterozygous for one nondeficient (class IV) and one deficient (class I–III variants) allele may display a normal or a deficient phenotype. It is therefore difficult to predict the phenotype of these individuals (**Supplementary Material** online (*G6PD* heterozygotes)).

IV) and one deficient (class I–III variants) allele; due to X-linked mosaicism, it is impossible to predict their activity based on genotype alone.

Almost 5% of the world's population is estimated to have G6PD deficiency, with nearly all of them carrying class II or III variants.[20] The average frequency of G6PD deficiency in malaria-endemic countries such as those in Asia and Africa is higher but variable, with a prevalence in certain population groups as high as 30% or more[3,20,21] (**Supplementary Tables S3–S5** online).

Genetic test interpretation

The *G6PD* gene is on the X chromosome (Xq28; ref. 2). Genotype results associated with G6PD deficiency may be reported as (i) hemizygous male (e.g., one class I–III variant allele), (ii) homozygous female (two identical deficient class I–III alleles with the same variant), (iii) compound heterozygous female (two different deficient class I–III alleles with different variants), and (iv) heterozygous female (one normal class IV allele and one deficient class I–III allele) (**Table 1**). The known inactivating or low-function variants (class I, II, and III variants) are provided in **Supplementary Table S1** online. If these variants are present, they may be interpreted as defined in **Table 1**, and in some cases a diagnosis of G6PD deficiency can be made on the basis of genotypic results. Hemizygous males, homozygous females, and compound heterozygous females are classified as either G6PD deficient or G6PD deficient with CNSHA (**Table 1**). For the rare male patients who have an extra X chromosome (i.e., Klinefelter's syndrome), *G6PD* genotype should be interpreted as if they are females.[22,23]

Determining G6PD phenotype in heterozygous females (one normal class IV allele and one deficient class I–III allele) is not possible based on genetic testing alone due to X-linked chromosome inactivation in females. This X-chromosome inactivation, which can happen in a variable percentage of somatic cells, inactivates either the normal or the low-activity allele and translates into heterozygous females having a mosaic of G6PD-normal and G6PD-deficient erythrocytes. The resulting overall enzyme activity will be variable because the ratio of the two types of red cells is highly variable and can change over time in the same individual.[2,3,24] Thus, G6PD activity in heterozygous females can potentially go the full range from being normal to being G6PD deficient, and thus heterozygotes may display a drug-induced AHA profile similar to that of homozygotes[2,19] (**Supplementary Material** online (G6PD Heterozygotes)). Thus, an enzyme activity test is needed to assign G6PD phenotype in heterozygous females.

Because most genetic tests do not comprehensively interrogate all variants associated with G6PD deficiency and because the phenotype of genotypically proven heterozygous females is unpredictable, most diagnoses of G6PD deficiency are currently made via tests of enzyme activity rather than genotype.[20] In males, the results of G6PD enzyme activity are usually clear cut, including in newborns, who tend to have higher activity than that observed in older children and adults.[25–27] The primary risk of misclassification in males is when there has been recent hemolysis (because G6PD in reticulocytes and in young erythrocytes is higher) or recent blood transfusion (because the transfused blood is likely to be G6PD normal); either or both may shift a G6PD-deficient enzyme level near to or even within the normal range. In females, there may be overlap in activity between G6PD homozygous normal and heterozygotes and between heterozygotes and homozygous deficient; there may be also more intrasubject variability in G6PD activity than in males (**Supplementary Material** online (*G6PD* Heterozygotes)). Universal neonatal screening programs for G6PD deficiency via the use of semiquantitative fluorescent spot test or quantitative enzyme activity assay have been instituted or proposed in areas with a high incidence of G6PD deficiency such as Asia, Europe, Africa, and the Middle East (**Supplementary Tables S3 and S4** online for frequencies in major racial/ethnic groups).

Available genetic test options

Commercially available genetic testing options change over time (**Supplementary Material** online (Available Genetic Test Options) and https://www.pharmgkb.org/views/viewGeneticTests.action). Genetic screening methods vary in their specificity and sensitivity.[20] The US National Newborn Screening Program routinely tests for G6PD deficiency via genotyping in the state of Pennsylvania and the District of Columbia, with a panel of five variants followed by confirmatory enzyme activity testing.[28] A serious limitation to genotype-only tests is that only a few of the known *G6PD* variants are usually included. This limitation may be overcome in the future by full sequencing of the gene. At the moment, the detection of class I–III variants in a male or of two class I–III alleles in a female is informative for predicting G6PD deficiency; on the other hand, a "negative" genotype result would not definitively rule out G6PD deficiency, and therefore an enzyme activity test would be needed to assess G6PD status.

Incidental findings

Hemolytic anemia. In G6PD-deficient individuals, AHA may be triggered by a number of different drugs (**Supplementary Table S6** online); future CPIC guidelines will be developed for other drugs affected by G6PD. AHA can also be triggered by exposure to certain chemicals, to fava beans, or to infection—all factors that cause increased oxidative stress in erythrocytes. Sometimes, for instance when a drug is given because of infection, it may be difficult to know whether the cause of AHA is the former or the latter.[1,2] AHA after the ingestion of fava beans (broad beans) in G6PD-deficient individuals is termed "favism" and can be fatal, mostly in children.[2,3] Individuals with a class I *G6PD* variant have a life-long disease (CNSHA) and in addition are at an increased risk of extravascular hemolytic episodes from exogenous triggering agents.[2]

Neonatal jaundice. Along with other factors, G6PD deficiency is associated with an increased risk of neonatal hyperbilirubinemia, which if left untreated can result in kernicterus, cerebral palsy, and death.[3,18,29] Risk may be further increased in those with the *UGT1A1*28* allele (rs8175347) associated with Gilbert's syndrome.[30]

Other clinical manifestations of G6PD deficiency. Numerous studies have investigated associations between G6PD activity and a variety of diseases; a critical analysis of evidence for these associations is beyond the scope of this guideline.

Other considerations

The extent of G6PD enzyme deficiency and clinical symptoms varies between and within individuals and is dependent on the type of *G6PD* genetic variant, the sex of the individual, the triggering agent, the presence of concurrent infection, and other inherited factors that may affect erythrocyte physiology (**Supplementary Material** online (Other Considerations)).[2,3,9,31]

DRUG: RASBURICASE

Background

Rasburicase (Elitek, Fasturtec, and Rasuritek) is a recombinant urate oxidase enzyme; it breaks down uric acid to hydrophilic allantoin and hydrogen peroxide.[6,7,32–34] Rasburicase is approved by the US Food and Drug Administration (FDA) for prophylaxis and treatment of hyperuricemia during chemotherapy in adults and children with lymphoma, leukemia, and solid tumors.[32] A pegylated form of urate oxidase, pegloticase (Krystexxa), is also FDA approved for the treatment of refractory gout.[35] Rasburicase has also been used in newborns who have high uric acid associated with kidney injury. Both rasburicase and pegloticase carry an FDA boxed warning and are contraindicated for use in patients with known G6PD deficiency.[32] The European Medicines Agency and Japan's Pharmaceuticals and Medical Devices Agency also contraindicate the use of rasburicase in patients with G6PD deficiency (http://www.pharmgkb.org/drug/PA10176) (http://www.pmda.go.jp/english/service/drugs.html).

Linking G6PD genetic variability to rasburicase-induced adverse reactions

Variation in G6PD enzyme activity has been related to a number of different *G6PD* genotypes (**Supplementary Table S1** online). Hydrogen peroxide is produced during the oxidation of uric acid to allantoin by rasburicase,[6] and this may cause hemolytic anemia (and possibly methemoglobinemia) after rasburicase administration in *G6PD*-deficient patients; this is supported by multiple clinical reports (**see Supplementary Table S7** online). Although much of the clinical data come from small case series and case reports, taken together and in view of the plausible underlying mechanism, many countries have drug label warnings that rasburicase use is contraindicated in those with G6PD deficiency (reviewed in refs. 1–5 and 9, (**Table 2**)).

Therapeutic recommendations

As stated above, rasburicase use is contraindicated by the FDA, the European Medicines Agency, and the Pharmaceuticals and Medical Devices Agency in those with G6PD deficiency[32–34] (see **Table 2**; **Figure 1**). If, on the basis of genotyping, a deficient status can be unambiguously assigned to a patient, that would be a sufficient contraindication to the use of rasburicase. However, due to the limitations of genetic testing (discussed above), in most cases it is necessary to perform G6PD enzyme testing to assign G6PD status.

The FDA recommends that patients at higher risk of G6PD deficiency, such as those with African or Mediterranean ancestry, be tested for G6PD deficiency before initiation of rasburicase.[32] However, it should be noted that patients of all ancestries may be G6PD deficient. The drug labels do not specifically mention genetic testing, but with the increased availability of genetic test results some patients may be diagnosed with G6PD deficiency preemptively; if so, such definitive results could be used to preclude prescribing of rasburicase and potentially other oxidative drugs even in the absence of G6PD enzyme activity results.

Pediatrics. Much of the evidence relating G6PD deficiency to rasburicase-induced hemolysis and methemoglobinemia was generated in neonates or children (**Supplementary Table S7** online), and thus these guidelines apply to neonates, children, and adults.

Recommendations for incidental findings

Patients with G6PD deficiency should be advised that they are at an increased risk of hemolysis after exposure to fava beans or to high-risk drugs or chemicals (**Supplementary Table S6** online), and that it is recommended to avoid such compounds (**Supplementary Material** online (Unsafe Drugs for G6PD Deficient Patients) and **Supplementary Table S6** online).

Table 2 Recommended therapeutic use of rasburicase in relation to G6PD phenotype

G6PD phenotype	Implications for phenotypic measures	Dosing recommendations for rasburicase	Classification of recommendations[a]
Normal[b]	Low or reduced risk of hemolytic anemia	No reason to withhold rasburicase based on G6PD status[b]	Strong
Deficient or deficient with CNSHA	At risk of acute hemolytic anemia	Rasburicase is contraindicated; alternatives include allopurinol[c]	Strong
Variable[b]	Unknown risk of hemolytic anemia	To ascertain that G6PD status is normal, enzyme activity must be measured; alternatives include allopurinol[c]	Moderate

CNSHA, chronic nonspherocytic hemolytic anemia.

[a]Rating scheme described in **Supplementary Material** online (see Strength of Recommendations). [b]A negative or inconclusive genetic test cannot be assumed to indicate normal G6PD phenotype; an enzyme activity test is needed to assign G6PD phenotype in such cases. [c]Allopurinol is associated with severe cutaneous reactions in the rare carriers of the *HLA-B*58:01* allele.[37]

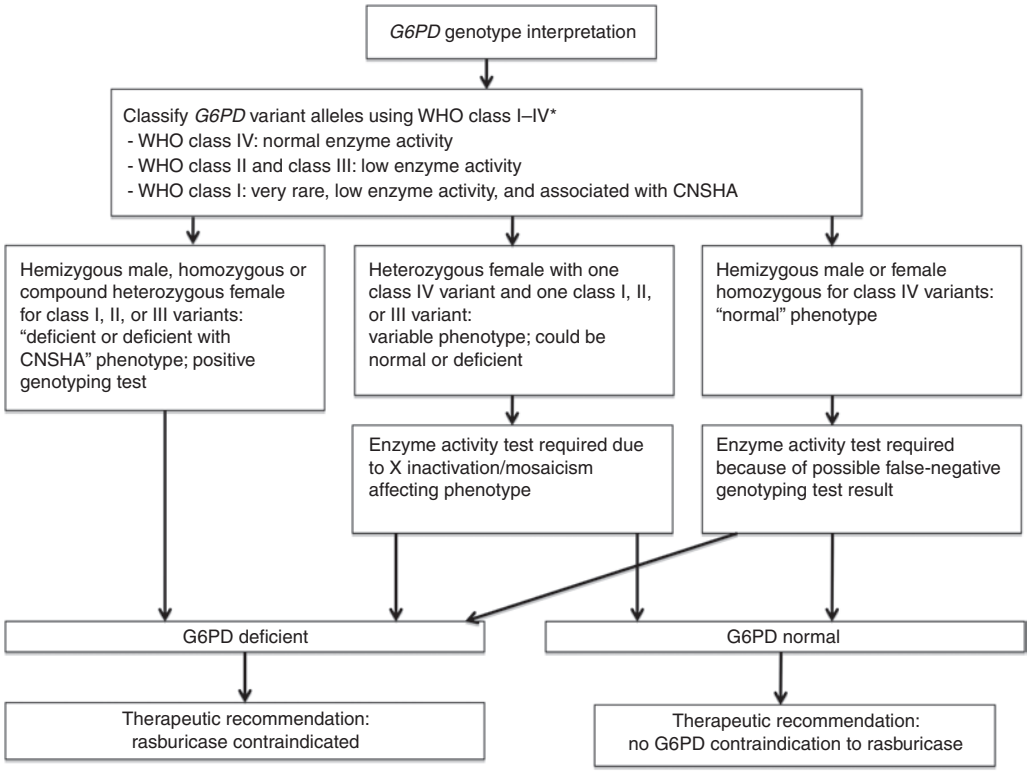

Figure 1 Workflow for interpreting *G6PD* genotype and for assessing the need for an enzyme activity test. *It should be noted that the class of a variant may have been assigned only by the clinical manifestations of a patient in which the variant was subsequently identified.[14] CNSHA, chronic nonsperocytic hemolytic anemia; WHO, World Health Organization.

Other considerations

Recommendations for the testing of other genetic markers are beyond the scope of this guideline. Agents known to induce or inhibit *G6PD* expression may also influence the risk of rasburicase-induced hemolysis.[36] Variation in the pharmacokinetics of rasburicase and dosage prescribed could also affect risk.[2]

POTENTIAL BENEFITS AND RISKS FOR THE PATIENT

Given the contraindication for using rasburicase in G6PD-deficient patients, for optimal decision making, clinical units treating tumor lysis syndrome should have access to G6PD status preemptively or access to enzyme activity results with rapid turnaround times. Administration of rasburicase to G6PD-deficient patients has resulted in cases of subsequent hemolytic anemia and methemoglobinemia, which can be fatal (**Supplementary Table S7** online). Of course, tumor lysis syndrome can itself be life-threatening, and alternative uric acid–lowering therapy, such as allopurinol, may not be as efficacious as rasburicase at lowering uric acid levels and has other potential side effects.[37,38] The risk of severe AHA and possible methemoglobinemia potentially caused by rasburicase versus the risk of tumor lysis syndrome complications if rasburicase is not used must be weighed against each other.

CAVEATS: APPROPRIATE USE AND/OR POTENTIAL MISUSE OF GENETIC TESTS

Several commercially available genetic tests screen only for some of the more common *G6PD* genetic variants. Therefore, any patient could have a rare, different, or previously unknown genetic variant; thus, a genetic test may have been reported as "negative," but the patient could nonetheless have G6PD deficiency.

CONCLUSION

Rasburicase is contraindicated in those with G6PD deficiency; unfortunately, the G6PD status of a patient is often unknown at the time rasburicase therapy is contemplated. If a genetic test result is preemptively available, it will be of use only if it establishes G6PD deficiency; otherwise, and in most cases, an enzyme test will be still required. In the future, as an increasing number of patients will have in their medical record a complete *G6PD* sequence, the percentage of informative genetic test results will increase, and the decision-making process regarding whether it is safe to use rasburicase will be accelerated and facilitated.

SUPPLEMENTARY MATERIAL is linked to the online version of the paper at http://www.nature.com/cpt

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CPIC guidelines reflect expert consensus based on clinical evidence and peer-reviewed literature available at the time they are written and are intended only to assist clinicians in decision making, as well as to identify questions for further research. New evidence may have emerged since the time a guideline was submitted for publication. Guidelines are limited in scope and

are not applicable to interventions or diseases not specifically identified. Guidelines do not account for all individual variation among patients and cannot be considered inclusive of all proper methods of care or exclusive of other treatments. It remains the responsibility of the health-care provider to determine the best course of treatment for the patient. Adherence to any guideline is voluntary, with the ultimate determination regarding its application to be made solely by the clinician and the patient. CPIC assumes no responsibility for any injury to persons or damage to property related to any use of CPIC's guidelines or for any errors or omissions.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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