HLA: flucloxacillin 4652

CI = confidence interval, CTCAE = common terminology criteria for adverse events, DILI = drug-induced liver injury, MHC = major histocompatibility complex = HLA and/or the HLA gene region, NS = non-significant, OR = odds ratio, RR = relative risk, S = significant

Disclaimer: The Pharmacogenetics Working Group of the KNMP formulates the optimal recommendations for each phenotype group based on the available evidence. If this optimal recommendation cannot be followed due to practical restrictions, e.g. therapeutic drug monitoring or a lower dose is not available, then the health care professional should consider the next best option.

Brief summary and justification of choices:
Although the mechanism of flucloxacillin-induced liver injury is not fully known, experimental data suggest the following mechanism. Flucloxacillin or flucloxacillin metabolites form a covalent bond with cellular proteins. Peptides derived from these modified proteins bind to HLA-B*5701 and are recognised on the cell surface as foreign by the immune cells, which triggers an immune response against cells containing flucloxacillin and/or its metabolites.
*HLA-B*5701*
HLA-B*5701 was found to strongly increase the risk for flucloxacillin-induced liver injury (OR = 36.6-100.0) (Nicoletti 2019 and Daly 2009). Because flucloxacillin-induced liver injury may be serious and rare cases of fatalities have been reported, the KNMP Pharmacogenetics Working Group concludes that action is needed for this gene-drug interaction (yes/yes-interaction).
Despite the strong association with HLA-B*5701, the incidence of flucloxacillin-induced liver injury among HLA-B*5701-positive patients is only 1-2 per 1000 individuals (Daly 2009). The incidence remains low if the 7-fold increase in patients > 70 years is taken into account (7-14 per 1000 individuals) (Nicoletti 2019). Therefore, a decision was made not to recommend an alternative for all HLA-B*5701-positive patients. Doctors are advised to monitor the liver function of HLA-B*5701-positive patients and to switch flucloxacillin to an alternative in the event of deterioration of liver function.
*HLA-B*5703*
HLA-B*5703 was found to strongly increase the risk for flucloxacillin-induced liver injury (OR = 79.2) (Nicoletti 2019). However, there is only limited evidence for an association. In the genome-wide association assay, there was no genome-wide significance for HLA-B*5703, only MHC-significance (HLA-region-wide significance). In addition, data were based on only two cases predicted to be positive for HLA-B*5703, with confirmation of HLA-B*5703 positivity for only one of the two cases by direct HLA typing. Finally, there is no confirmation from a second (independent or extended) case-control group. For these reasons, the KNMP Pharmacogenetics Working Group decided that, at this moment, there is not enough evidence for a gene-drug interaction and not enough cause for inclusion of this gene-drug combinations in the electronic decision support systems and/or the KNMP Kennisbank.
For HLA-B*5701, you can find a detailed overview of the observed clinical effects in the background information text of the gene-drug interaction on the KNMP Kennisbank. You might also have access to this background information text via your pharmacy or physician electronic decision support system.

Recommendation concerning pre-emptive genotyping, including justification of choices:
The KNMP Pharmacogenetics Working Group considers genotyping before starting flucloxacillin to be beneficial for drug safety. It is advised to consider genotyping these patients before (or directly after) drug therapy has been initiated to guide drug selection.
The clinical implication of the gene-drug interaction scores 5 out of the maximum of 10 points (with pre-emptive genotyping considered to be beneficial for scores ranging from 3 to 5 points) (see also the clinical implication score tables at the end of this risk analysis):
HLA-B*5701 increases the risk of flucloxacillin induced liver injury. The SmPC states that flucloxacillin-induced liver function impairments can be fatal (severity code F, corresponding to CTCAE grade 5). This results in the maximum of 2 points for the first criterion of the clinical implication score, the clinical effect associated with the gene-drug interaction (2 points for CTCAE grade 5).
Two studies showed HLA-B*5701 to increase the risk of flucloxacillin-induced severe liver injury (CTCAE grade 3). This results in 2 out of the maximum of 3 points for the second criterion of the clinical implication score, the level of evidence supporting an associated clinical effect grade ≥ 3 (2 points for two publications with level of evidence score ≥ 3).

Wing 2017 reports the highest incidence of flucloxacillin-induced liver injury in the UK to be 110.5 per 100,000 in those aged > 70 years who have received 2 or more prescriptions (Wing K et al. Quantification of the risk of liver injury associated with flucloxacillin: a UK population-based cohort study. J Antimicrob Chemother 2017;72:2636-46). Nicoletti 2019 showed the prevalence of HLA-B*5701 in flucloxacillin-induced liver injury to be 82% in a population of North-European descent. This would amount to an incidence of 90.6 cases of liver injury due to the HLA-B*5701-flucloxacillin interaction per 100.000 patients aged > 70 years who have received 2 or more prescriptions. So, even in this high risk group the incidence is below 1 per 1000, indicating that more than 1000 patients should be screened for HLA-B*5701 to prevent one case of flucloxacillin-induced liver injury. This results in 0 of the maximum of 3 points for the third criterion of the clinical implication score, the number needed to genotype (NNG) to prevent one clinical effect grade ≥ 3 (only points for NNG ≤ 1000).
The Summary of Product Characteristics (SmPC) of flucloxacillin mentions HLA-B*5701 to increase the risk of flucloxacillin-induced liver injury, but does not mention HLA-B*5701 as a contra-indication and indicates that pre-emptive genotyping is not recommend. This results in 1 out of the maximum of 2 points for the fourth and last criterion of the clinical implication score, the pharmacogenetics information in the SmPC (1 point for at least one genotype/phenotype mentioned in the SmPC, but not mentioned as a contraindication and no recommendation to genotype).

Source	Code	Effect	Comments
ref. 1 Teixeira M et al. Flucloxacillin-induced hepatotoxicity - association with HLA-B*5701. Rev Assoc Med Bras 2020;66:12-7. PMID: 32130375.	2 B*5701: D	A 74-year old man was hospitalised with moderate to severe mixed hepatocellular and cholestatic hepatotoxicity after having received an 8-day cycle of flucloxacillin 500 mg 3 times daily for erysipelas twice, 3 months and 2 weeks earlier. The patient presented with asthenia, anorexia, nausea, abdominal discomfort, and mild fever. Plasma concentrations of liver enzymes and fatty acids were elevated: aspartate aminotransferase 8.1x the upper limit of normal (ULN), alanine aminotransferase 15x ULN, fatty acids 3x ULN, gamma-glutamyl transferase 13.3x ULN, total bilirubin 2.8x ULN, and direct bilirubin 5.5x ULN. The inflammation marker C-reactive protein was elevated (7.9x ULN), but leukocytes were not. The anatomopathological findings showed signs that may be associated with a poor prognosis, with the need for liver transplantation and higher mortality, namely the presence of microvesicular steatosis, hepatocyte necrosis, cholangial cholestasis, and duct proliferation. The patient had a history of monoclonal gammopathy under investigation, did not use chronic medication, and used 24 g of alcohol per day. Renal function was normal. Autoimmunity, hepatotropic viruses, chronic liver disease, and amyloidosis were excluded as causes. The Maria and Vitorino scale showed possible causality of flucloxacillin (13 points), and the Rucam scale likely causality (8 points). The patient was HLA-B*5701 positive. During hospitalization plasma concentrations of aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase, and alkaline phosphatase decreased (although the latter with variations). However, plasma concentrations of bilirubin worsened, reaching 20.1x ULN for total bilirubin and 44.6x ULN for direct bilirubin, with development of jaundice, pruritus, and choluria, without coagulopathy or encephalopathy. The presence of jaundice concomitant to the hepatotoxicity scenario is associated with an approximately 10% likelihood of risk of progression to fatal liver failure. Once hepatotoxicity was confirmed, treatment with ursodeoxycholic acid was started at 1,500 mg/day, in the 21st day of hospitalization. After flucloxacillin had been suspended for four months, there was clinical and analytical improvement with aspartate aminotransferase 1.4x ULN, alanine aminotransferase 1.9x ULN, fatty acids 2.2x ULN, gamma-glutamyl transferase 3.9x	Authors' conclusion: "The authors present this case to remind the possibility of moderate/ severe drug-induced liver injury to flucloxacillin, an antibiotic commonly used in clinical practice and association with the HLA-B * 5701 allele reported in the literature."

ref. 1, continuation		ULN, total bilirubin 1.2xULN, and direct bilirubin 2.1x ULN. About a year later, there was still a slight elevation of fatty acids (1.1x ULN) and gamma-glutamyl transferase (2.0x ULN) with the other liver enzymology values within the normal range. After 18 months, only gamma-glutamyl transferase remained high, suggesting chronic bile duct injury.	
ref. 2 Nicoletti P et al. Drug-induced liver injury due to flucloxacillin: relevance of multiple human leukocyte antigen alleles. Clin Pharmacol Ther 2019;106:245-53. PMID: 30661239.	4 B*5701: D B*5703: D	A genome-wide association study among 197 cases of flucloxacillin-induced drug-induced liver injury (DILI) and 6,835 controls (all Northern-European ethnic origin) was performed. 51 of the cases were also included in the study of Daly 2009. The strongest association in the genome-wide association study was with HLA-B*5701 (OR = 36.62; 95% CI = 26.14-51.29; P = $2.67x10^{-97}$). Haplotype analysis showed that HLA-B*5701-containing haplotypes confer risk, whereas B*5701-negative haplotypes seem to be protective, suggesting that B*5701 and no other allele within the haplotype is the main risk factor. 82% of the cases was HLA-B*5701 positive. Reciprocal conditional analyses on the originally identified associated SNP rs2395029 and HLA-B*5701 demonstrated that HLA-B*5703 was an MHC-significant independent risk allele (OR = 79.21; 95% CI = 13.57-462.4; P = $1.2x10^{-6}$). 2 cases (1%) were predicted to be positive for HLA-B*5703. This was confirmed for one of the two cases by direct HLA typing. No strong association of other HLA molecules was observed. Flucloxacillin being the cause of DILI was highly probable in 43% of cases, probable in 46% and possible in the remaining 11% (scores of respectively > 8, 6-8 and 3-5 on the scale of the Council for International Organizations of Medical Science (also called Roussel Uclaf Causality Assessment Method (RUCAM) scale)). This indicates that relevant comedication was excluded for the majority of patients. NOTE: The cases used flucloxacillin for an average of 10 days and liver injury was diagnosed after an average of 23.4 days. NOTE: Analysis with a subset of 380 controls showed sex, age, and HLA-B*57 to be associated with DILI in a univariate model, but in a multivariate analysis model only age above 70 years and HLA-B*57 remained significant. These data suggested that for those older than 70 years there was a sevenfold increase in DILI risk. Wing 2017 indicates that in the UK the incidence of DILI is 8.5 per 100,000 people prescribed flucloxacillin, increasing to 35 per 100,000 in those receiving 1 consecutive prescription and 110.5 per 100,000 in those aged > 70 years who have received 2 or more prescriptions (Wing K et al. Quantification of the risk of liver injury associated with flucloxacillin: a UK population-based cohort study. J Antimicrob Chemother 2017;72:2636-46).	Authors' conclusion: "HLA-B*57:01 was the major risk factor. HLA-B*57:03 also showed an association. Within the HLA-B protein sequence, imputation showed valine97, common to HLA-B*57:01 and HLA-B*57:03, had the largest effect."
ref. 3 Vera JH et al. The safety of flucloxacillin in HIV-infected patients with positive HLA-B*5701 genotype. Aids 2013;27:484-5. PubMed PMID:	2 B*5701: AA	Data from 10 carriers of HLA-B*5701 treated with flucloxacillin (duration 5-14 days, total dose 10-28 g, 9 men, median age 51 years) were analysed retrospectively over a period of 3.5 years. No clinical or biochemical indication for drug-induced liver injury (DILI) was found between 1 and 90 days after initiation of flucloxacillin. The authors reported that the previously identified risk factors for flucloxacillin-induced DILI (usage period exceeding 14 days, age older than 55 years and female sex) were absent or largely absent in the treated patient group.	Authors' conclusion: "For HLA-B*5701 positive individuals in whom other risk factors are present, alternative antibiotics to flucloxacillin could be considered. However,

23032409. Phillips EJ et al. HLA-B*5701 and flucloxacillin associated drug-induced liver disease. AIDS 2013;27:491-2. PubMed PMID: 23291545.		Comments by Philips et al: Given the low prevalence of flucloxacillin-induced DILI (prevalence 8.5/100,000 in the total population and 1-2/1000 in HLA-B*5701-positive individuals), the Vera et al. study has insufficient power to draw definitive conclusions about the safety of flucloxacillin in HLA-B*5701-positive individuals.	given that most clinicians are comfortable and confident using flucloxacillin, careful monitoring of liver function parameters would seem to be a prudent alternative based on these initial data."
ref. 4 Daly AK et al. HLA-B*5701 genotype is a major determinant of drug-induced liver injury due to flucloxacillin. Nature Genetics 2009;41:816-9. PubMed PMID: 19483685.	4 B*5701: D	A genome-wide association study among 58 cases of flucloxacillin-induced drug-induced liver injury (DILI) and 282 controls (all Northern-European ethnic origin) identified the highest association with an SNP only present (complete linkage disequilibrium) in carriers of HLA-B*5701 ($p = 8.7 \times 10^{-33}$; OR = 45; 95% CI: 19.4-105) (S). 84% of the patients were carriers of this genotype versus 11% of the controls. HLA-B*5701 genotyping in the 51 cases using only flucloxacillin and a new group of controls (n = 64) confirmed the association where the HLA-B*5701 allele was associated with an 80-fold elevated risk of developing flucloxacillin-related DILI (OR = 80.6; 95% CI: 22.8-284.9) (S). HLA-B*5701 was not associated with the severity of the DILI. The association was confirmed in a new set consisting of the 7 remaining cases and 16 new cases (OR = 100.0; 95% CI: 20.6-485.8) (S). The remaining 7 cases had used amoxicillin/clavulanic acid alongside flucloxacillin, which can also trigger DILI. The authors reported that the incidence of DILI among HLA-B*5701-positive patients receiving flucloxacillin is only 1-2 per 1000 despite the strong association with HLA-B*5701. NOTE: The cases used flucloxacillin for an average of 11.1 days and liver injury was diagnosed after an average of 23.4 days.	Authors' conclusion: "Our study of genetic association with drug induced liver injury (DILI) linked to a licensed drug done on a genome-wide scale shows that HLA-B*5701 is the main common genetic risk factor for flucloxacillin DILI and provides suggestive evidence for additional contributions from other loci."
ref. 5 SmPC Floxapen (flucloxacillin) 15-04-21.	0 B*5701: F	<u>Adverse events/Pharmacodynamics</u>: There are indications that the risk of flucloxacillin-induced liver injury is increased for persons carrying the HLA-8 * 5701 allele. Despite this strong association, only 1 of 500-1000 carriers will develop liver injury. As a consequence, the positive predictive value of the HLA-8 * 5701 allele for liver injury is very low (0.12%) and routine testing for this allele is not recommended. <u>Adverse events</u>: Liver function impairments can be severe, and under rare circumstances a fatal outcome is reported. The majority of reports of fatal outcome concerned patients over 50 years of age and patients with severe underlying disease.	

Risk group	treatment duration exceeding 14 days, older age (older than 70 or 55 years), female sex

Comments:

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Date of literature search: 22 October 2021.

Genotype	Code	Gene-drug interaction	Action	Date

KNMP Pharmacogenetics Working group decision	HLA-B*5701	4F	Yes	Yes	15 November 2021

Mechanism:
Although the mechanism of flucloxacillin-induced liver injury is not fully known, experimental data suggest the following mechanism.
Flucloxacillin or flucloxacillin metabolites form a covalent bond with cellular proteins. Peptides derived from these modified proteins bind to HLA-B*5701 and are recognised on the cell surface as foreign by the immune cells, which triggers an immune response against cells containing flucloxacillin and/or its metabolites.

Clinical Implication Score:

Table 1: Definitions of the available Clinical Implication Scores

Potentially beneficial	PGx testing for this gene-drug pair is potentially beneficial. Genotyping can be considered on an individual patient basis. If, however, the genotype is available, the DPWG recommends adhering to the gene-drug guideline	0-2 +
Beneficial	PGx testing for this gene-drug pair is beneficial. It is advised to consider genotyping the patient before (or directly after) drug therapy has been initiated to guide drug and dose selection	3-5 +
Essential	PGx testing for this gene-drug pair is essential for drug safety or efficacy. Genotyping must be performed before drug therapy has been initiated to guide drug and dose selection	6-10 +

Table 2: Criteria on which the attribution of Clinical Implication Score is based

Clinical Implication Score Criteria	**Possible Score**	**Given Score**
Clinical effect associated with gene-drug interaction (drug- or diminished efficacy-induced)		
• CTCAE Grade 3 or 4 (clinical effect score D or E)	+	
• CTCAE Grade 5 (clinical effect score F)	++	++
Level of evidence supporting the associated clinical effect grade ≥ 3		
• One study with level of evidence score ≥ 3	+	
• Two studies with level of evidence score ≥ 3	++	++
• Three or more studies with level of evidence score ≥ 3	+++	
Number needed to genotype (NNG) in the Dutch population to prevent one clinical effect grade ≥ 3		
• 100 < NNG ≤ 1000	+	
• 10 < NNG ≤ 100	++	
• NNG ≤ 10	+++	
PGx information in the Summary of Product Characteristics (SmPC)		
• At least one genotype/phenotype mentioned	+	+
OR		
• Recommendation to genotype	++	
OR		
• At least one genotype/phenotype mentioned as a contra-indication in the corresponding section	++	
Total Score:	10+	5+
Corresponding Clinical Implication Score:		Beneficial