I would like to start by thanking John Senior and the organizers for inviting me. Thank you very much. I appreciate this very interesting meeting.

I just would like to mention, before I go into this drug-induced autoimmune hepatitis, the features that Jack Uetrecht mentioned before of the immunoallergic reactions. When I was working in Sweden, where I spent almost 20 years, we analyzed reports that came to the Swedish Adverse Drug Reactionary Committee from physicians in Sweden.
And cases of disulfiram and others, this is a very well-documented hepatotoxic drug. And we found among these patients that were reported, eight died. This is in accordance with Hy's rule, about 10 percent mortality.
To our surprise, we found two different phenotypes histologically. This phenotype with immunoallergic features with hepatic and peripheral eosinophilia. You can see in the liver lobe that there are numerous eosinophils, which is an inflammatory infiltrate. These patients all had a very favorable outcome. They all survived.
Whereas, with a centrilobular dropout of necrosis, this feature not surprisingly lead to a very bad outcome with death from liver failure or transplantation.
And we looked at report from different registers around the world and it turned out to be true that, for example, in the Spanish hepatitis registry, patients who died very, very rarely had any immunoallergic features. It is interesting.
We also looked at all the drugs that are very well documented, and we found the same thing. There was a lot of difference between those who had immunoallergic features and those who did not, in terms of severity of liver disease and prognosis. So, this was truthful for all these drugs.
So, all the time you present something that is new, people become skeptical, for good reason.
So, I was very happy to see that this could be reproduced in another cohort and this was a study from India, where tuberculosis in India is a big health problem and will still haven't come up with all the drugs that do not include isoniazid. And a lot of children in India die from isoniazid-induced liver injury. And he looked at patients, actually children, with drug-induced liver injury and he found that those with hypersensitivity have much better outcome. Those who had hypersensitivity features have no mortality, whereas, this was present in almost 50 percent of those without these features. I would just like to mention this because this is an immunoallergic feature.
So, coming back to this autoimmune hepatitis, Dr. Czaja has mentioned, this can be defined as an adverse immune response to proteins within the liver, initiated by a drug, clinically similar to autoimmune hepatitis.

As was shown and mentioned before by Dr. Czaja, tienilic acid was a prototype in the '80s or '70s for this type of reaction. This has been removed from the market, I think. And that the reactive metabolites created through hepatic metabolism of some drugs have been shown to bind to cellular proteins such as cytochrome P450. These can then be recognized by the immune system as neoantigens.

Nitrofurantoin, minocycline, α-methyl dopa, hydralazine have all been well documented as drug-induced AIH. More recently TNF-α antagonists and statins.

Drug-induced AIH

* An adverse immune response to proteins within the liver initiated by a drug, clinically similar to autoimmune hepatitis

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So, coming back to this autoimmune hepatitis, Dr. Czaja has mentioned, this can be defined as an adverse immune response to proteins within the liver, initiated by a drug. And this is similarly clinically and biochemically and also histological to idiopathic autoimmune hepatitis.

As was shown and mentioned before by Dr. Czaja, tienilic acid was a prototype in the '80s or '70s for this type of reaction. This has been removed from the market, I think. And that the reactive metabolites created through hepatic metabolism of some drugs have been shown to bind to cellular proteins such as cytochrome P450. And this can be recognized by the immune system as neoantigens.
There are some drugs that are particularly associated with this type of liver injury: nitrofurantoin, still in wide use; minocycline, alpha-methyl dopa, and hydralazine. More recently, TNF-alpha antagonists and statins have been implicated in this type of liver injury.

So, this has been caused by drugs. There are limited data comparing these patients with other patients with autoimmune hepatitis.
So, when I spent time at the Mayo Clinic a few years ago, I looked for these cases in the Mayo Clinic diagnosed medical intakes and we searched for the text in the medical records. Not anywhere in the world, and not even at this fine clinic, can we trust the diagnoses that doctors make. Isn't that right? (Laughter.)

So, this is the way to look for diagnosis. Look for it in the text and then screen to see if this terminology is present in the text, we can look for this case and this can be a differential diagnosis. It can be a history or family history and so on. So then we can come up with a number of good cases.

And in this part, we excluded overlap syndromes with PBC and PSC and decompensated liver cirrhosis.
So, among 261 patients with well-characterized autoimmune hepatitis, we were able to find 24 drug-induced autoimmune hepatitis, mostly due to nitrofurantoin and minocycline in this series.
Interestingly, a very similar proportion of those with drug-induced autoimmune hepatitis and idiopathic had antinuclear antibodies and smooth muscle antibodies. There was no difference. And interestingly, the histological grade and stage were similar in these two groups, but none of the drug-induced autoimmune hepatitis had cirrhosis at the baseline; whereas, this was present in 20 percent of the matched autoimmune hepatitis cases.

Results

* Histological grade and stage were similar in patients with DIAIH vs. AIH but none of the DIAIH patients had cirrhosis at baseline; this was present in 20% of matched AIH cases.

* Liver imaging was normal in all minocycline cases. 8/11 (73%) of nitrofurantoin patients had abnormalities on hepatic imaging (mainly liver atrophy) a finding seen in only 8/33 (24%) of a random sample of the rest of the AIH group (p=0.0089).
We looked at liver imaging because they found that this was abnormal in the nitrofurantoin patients. This was normal in all the minocycline cases. We saw that liver atrophy and confluent fibrosis centrally was characteristic for the nitrofurantoin-induced autoimmune hepatitis. See atrophy of the liver and here is the confluent fibrosis.
We looked also at the corticosteroid responsiveness. This was very similar but the only difference we could identify was when the immunosuppressive drugs were discontinued. When this was tried, physicians -- there is a difference between the doctors how eager they are to change anything. And if they wanted to discontinue this immunosuppression, when this was tried, this was successful in all these cases and no relapses. Whereas, during this follow-up in the autoimmune hepatitis group, 65 percent had a relapse.

Results

* Corticosteroid responsiveness was similar in DIAIH and the AIH patients.

* Discontinuation of immunosuppression was tried and successful in 14 DIAIH cases, with no relapses (0%) whereas 65% of the AIH patients had a relapse after discontinuation of immunosuppression (p<0.0001).
So, we, from this series conclude a significant proportion, between nine and ten percent of patients with autoimmune hepatitis have drug-induced autoimmune hepatitis. And these groups had similar clinical and histological patterns. But at least, according to our data, they do not seem to require long-term immunosuppressive therapy. So, I think that the DILIN network is now working on a further analysis of their cases with drug-induced autoimmune hepatitis. This may involve minocycline, hydralazine, and alpha methyl dopa. And I think an abstractor from this work will be presented at the ESIL meeting.
As Jack mentioned before, TNF-alpha antagonists have been found to be associated with drug-induced liver injury (DILI) with numerous case reports. The largest series included 6 patients (from the US) with additional 28 cases from the literature (Ghabril et al. Clin Gastro Hepatol 2013).

Little is known about the absolute risk of liver injury with these drugs. And, in Iceland, this is a small country, but we have advantages that we can cover the whole country. We can trace all these patients and look for them where they hide. And they cannot leave the island unless we test them.
So, we found in a recent paper that an absolute risk of DILI associated with infliximab was one out of 148 treated patients. This was over a two-year period in a prospective study. And we because we have the Director of Medicine who doesn't have a medicine registry, all prescriptions, both within hospital and outside hospital are registered, so we could match these patients with the registry. We come up with these figures.
So, we wanted to look both before this two-year prospective study and after for a five-year period to look for if this is true also for the paired outside the study in a population-based study.

So, we tried to identify all patients with suspected drug-induced liver injury treated with TNF-alpha antagonists in Iceland and we analyzed the clinical characteristic and features of autoimmunity.
So we could, during this five-year period, come up with 11 patients. And much are females and a total of nine patients have been treated with infliximab. And I just think that this reflects the use of these drugs. Infliximab was the first TNF-alpha antagonist and most widely used still. Only two of these patients have inflammatory bowel disease; whereas, mostly had rheumatological conditions.

**Results**

* 11 patients, 8 females (73%), mean age 46 years (3) were found during the study period to have at least possible relationship according to RUCAM.

* A total of 9 patients (82%) had been treated with infliximab, one with etanercept and one with adalimumab.

* Two patients (18%) had Crohns disease or ulcerative colitis whereas seronegative rheumatoid arthritis (RA) (n=4), seropositive RA (n=2), spondylarthritis (n=1), psoriasis arthritis (n=1) and psoriasis (n=1).
And during this period, over 1,076 patients had been started on infliximab. We could even find a higher proportion patients develop DILI. One of 120 patients treated with infliximab developed this kind of liver injury.

**Absolute risk of DILI associated with TNF-alpha antagonists**

- During 2009-2013, overall 1076 patients had been started on infliximab leading to a DILI episode in:
  - 1/120 patients treated with Infliximab,
  - 1/270 treated with adalimumab
  - 1/430 treated with etanercept.
Results

* Overall 4/11 (36%) had jaundice at diagnosis

* 8 had hepatocellular, two cholestatic and one mixed pattern of liver injury.

* Max ALT 704 (102), range: 169-1658

* AST 503 (115), range: 91-1375

* ALP 261 (76), range: 71-916

* Bilirubin 38 (11), range: 10-100

So, just more than a third had jaundice, and the particular phenotype was hepatocellular with very high ALT and AST and features of autoimmune hepatitis or autoimmunity.
What we wanted to do that nobody had done before was to match these patients with controls on TNF-alpha antagonist not to develop disease, not develop this reaction. And we matched these patients by age and gender, as well as the indication for which the drug was given. I think this is very important because these patients, mostly those with rheumatological conditions, have immune-dysregulation. So, it is important to match or think about the immune features before or at baseline. And we didn't find any difference between these groups except for the presence of methotrexate. This is a widely used drug in rheumatology. And also we looked at the ANA positivity prior to TNF-alpha therapy. There was no difference in those who have been tested.

And it has also been taken into consideration that some of these drugs induced ANA, although, in some of these patients, they don't necessarily develop autoimmune hepatitis. But among those who developed liver injury, a significantly less proportion of patients were on methotrexate, whereas in the controls, this was more frequent. So, in this context it seems to protect against this type of liver injury.
Liver histology

* Liver biopsies were performed in 5/11 (46%)
* Severe acute hepatitis pattern (n=3),
* Acute and chronic hepatitis pattern (n=1)
* Canalicular cholestasis (n=1)

We have liver biopsies on approximately half, mostly hepatitis.
And you can see a patient, 40-year-old woman who developed dense inflammatory infiltrate yet, you see apoptopic cell here and these features might look like autoimmune hepatitis. What do you say, Albert?

DR. CZAJA: Yes.

And these are the figures that she presented with, and for a two-month period her ALT doesn't seem to go down. And there was a problem with the biopsy. She had elevated APTT and we have to look for and explain that. So, we didn't do the biopsy until two months after the presentation. And the biopsy was, as I showed before. And she had positive ANA, immunoglobulal, et cetera. She started steroids and became rapidly improved, clinically and biochemically. She is now off immunosuppression and for a follow-up of two years, she hasn't had a relapse.
ALT before and after steroids in a 40 year old female with Infliximab DILI
This is another type of reaction, which also showed ANA. This patient was symptomatic presented approximately with ALT 800. And as you see here, when you follow the patient, she spontaneously goes down and no immunosuppression was required.
So, half of these patients were treated with steroids and this could be discontinued in all where we tried but in one patient, he is still on treatment. And that is a decision of the responsible physician to do so.
We found infliximab was more often associated with DILI than other TNF-alpha antagonists and autoimmune features are frequently in these patients and required steroids in approximately half of these patients. But despite this, the overall prognosis is favorable. So, the vast majority do not need steroid, long-term. And what was important was that when we tried other TNF alpha antagonists, it was always safe.
So, I am just turning a little bit about, turning my attention to this association between drug-induced liver injury and autoimmune hepatitis. In a long-term follow-up of patients who have concomitant jaundice leading to hospitalization, AIH developed in 5/23 (22%) patients (all females) after the initial event over a mean period of 6 years (Bjornsson and Davidsdottir. J Hepatology 2009)
And it has also been shown that ANA can be detected after DILI and later on during follow-up.

DILI and AIH

* In a Japanese study, ANA was detected after DILI in 6 patients and 5/6 (83%) were females (Ohmoto K, et al. Drug-induced liver injury associated with antinuclear antibodies. Scand J Gastroenterol. 2002;37:1345–6.)

* In another study, 7 cases diagnosed as DILI, but features of AIH became apparent later despite discontinuation of the drug. Interestingly, ANA titers and immunoglobulin (Ig) G levels increased during the course. (Sugimoto K. Seven cases of autoimmune hepatitis that developed after drug-induced liver injury. Hepatology. 2011;54:1892–3.)
Interestingly, in the Spanish hepatotoxicity registry, nine out of 700 patients or 1.2 percent had evidence of two DILI episodes caused by different drugs. And an interesting finding was that four out of these nine cases developed drug-induced autoimmune hepatitis in the second episode. This clearly exceeds the chance of association of this liver injury phenotype in the Spanish DILI registry’s general patient cohort.


In most cases drug-induced autoimmune hepatitis have developed injury associated with drug intake and autoimmune features.
And the question is if it is adequate for diagnosis to have the drug intake and an elevation of autoantibodies. Probably not, because some drugs can lead to development of autoantibodies. Maybe it is important to also take into consideration the history, if this preceded the symptoms of liver injury.
And we often need to do a liver biopsy, particularly those with a persistent liver injury. And when this was done in a subgroup analysis of the use of liver biopsy and distinguishing autoimmune hepatitis and drug-induced liver injury, we found that the severity of inflammation and fibrosis was similar but marked fibrosis was very much -- was only seen in patients with classical autoimmune hepatitis, as I mentioned earlier.

**Diagnosis**

* HISTOLOGY: A subgroup analysis demonstrated that severity of inflammation and fibrosis and the frequency of what have been considered AIH-specific findings were comparable between DIAH and AIH.

* Marked fibrosis (Ishak score >4) was however only seen in patients with classical AIH and not in DIAIH cases.

For management, we need to identify the role of drug. I am going to skip slides here a little bit because of the time.
And I think some patients do not require immunosuppression, as with the second patient I showed you. And of those who do not normalize their liver test, we need steroids. But the question is: how long do we require the immunosuppression?

There has been success with drugs in most cases that have been reported but I could only come up with three cases where this has not been possible. Of course, you need to follow the patient.
I just want to finish with an email I received recently from Turkey. I am a pediatric surgeon. I have a 17-year-old daughter. She has been diagnosed with type II AIH. I have doubts about the diagnosis, the treatment protocol and duration of treatment. Read your article: Drug-induced AIH etc. we need your suggestion and advice.

My daughter has not any complaints. Physical examination signs are normal. Weight is 46 kg. Past medical history was significant for chronic urticaria for 18 months and acne vulgaris. August 5, 2014. She was prescribed Isotretinoin (Roaccutane) 30 mg/day by dermatologist for acne vulgaris.
My daughter had no complaints; physical examination was normal. She had a problem with acne vulgaris. And on the fifth of August 2014 she was prescribed Rosaccutane, isotretinoin for acne vulgaris. And these were the liver test prior to treatment with Roaccutane AST 36, ALT 43, slightly above the limit. But after a month, ALT goes up to 140 and -- ALT is 91 and two weeks' later it is 141. And she has ANA positivity and also anti-LKM. Other causes are excluded.
And the histopathology showed portal and periportal plasma cell-rich inflammation and marked lobular necro inflammatory activity. Staging: Fibrosis 1/6.

January 29, 2015. The diagnosis of type II Autoimmune Hepatitis was made and treatment with prednisone 60 mg daily was begun. Azathioprine 50 mg daily will be added one week later and prednisone will be gradually decreased to 15 mg daily. The treatment will be continued for two years.

And the histopathology showed portal and periportal plasma, accelerates inflammation, fibrosis 1/6. And this was the suggested treatment: prednisone 60 milligrams daily for -- it started with 60 milligrams daily with tapering and also azathioprine at the same time. This was supposed to go on for two years.
And we questioned the diagnosis, diagnosis Type 2 AIH or drug-induced hepatitis? Was the treatment protocol suitable? How long should the treatment be, et cetera, et cetera?

What to do?

“What do you think about the diagnosis? Is the diagnosis Type II Autoimmune Hepatitis or Drug-Induced Autoimmune Hepatitis?

Is the treatment protocol suitable for seventeen years old girl?

How long should be the duration of the treatment?

What could you suggest for treatment and duration of treatment?”
So, I don't think that drug has been associated with drug-induced autoimmune hepatitis but for the first I don't think that a 60 milligram. That is quite a high dose. Maybe 20 or 30. What do you think?

Conclusions

* In a patient with a high clinical suspicion of DILI with positive autoantibodies and/or with the syndrome of DIAH, immunosuppression is indicated if aminotransferases remain elevated despite discontinuation of the suspected drug.

* Discontinuation of immunosuppression in patients with DIAIH when attempted is usually successful and immunosuppression is rarely required long-term in these patients.