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Hemophagocytic lymphohistiocytosis (HLH) (Held in collaboration with SIOPe)

Dr Hutter: Welcome, everybody. I hope you can soon see the presentation. So my name is Caroline Hutter, I'm from the St. Anna Children's Hospital, Vienna I'm here today with Andishe Attarbaschi, my colleague from the same hospital and we'll discussed today HLH. So the goal of this session is to discuss how to diagnose the disease, review the pathogenesis and discuss some of the treatment options which is sometimes not so easy. So, HLH is uncontrolled inflammation that leads to Fever, Cytopenia and Splenomegaly, and the name comes from the Hemophagocytosis. You can sometimes see and that's on the picture you can see here, where you have macrophages that have eaten some of the erythrocytes. So hence the name Hemophagocytosis. Just be careful Hemophagocytosis doesn't mean that we have Hemophagocytic Syndrome. Hemophagocytosis is something you can often see during a chronic inflammation. So it's not very specific for the disease. What is HLH? So it's a life-threatening systemic hyper-inflammatory syndrome. And it's characterized by a few clinical symptoms, like a fever, cytopenia, coagulopathy and the elevation of some biomarkers. I will go into this later. You have to be a bit careful, the terminology is a bit of a minefield, because people are talking about primary and secondary HLH and there's a lot of discussion whether this should be dropped or not to discern between primary and secondary. There are two terminals HLH spectrum disorder, and we also have the MAS a macrophage activating syndrome. I we'll talk about this later though. So I think it's important from the beginning just to bear in mind the phenotype is caused by quite diverse underlying molecular diagnosis. And it's not a distinct disease. HLH is important. It's a very dangerous disease with a very high lethality. So this data are from children, but you can see that more or less, if you don't recognize it the child will die. Less than 10% of patients who are not treated survive the disease. And the majority of deaths occurs in the first eight weeks. So this means that you don't have a lot of time often to diagnosis it. And even if you are treated, the outcome is not very good still. How do you diagnose HLH? I think it is something that is very difficult. And the initial experience condition is not straightforward. You can see one is a familial disease, non-genetic defects. So this is something that in the first instance isn't very helpful, because of course, you don't start to sequence any patients. So this is more helpful in the setting where you have a sibling that has been affected, or if you want to make sure that the diagnosis you have already suspected is there or not. So the clinical and laboratory criteria are quite unspecific. So it's more of a constellation of these different findings that helps you to diagnose HLH. So when there is fever above 38.5, it needs to be there have a find of the splenomegaly and cytopenia in at least two cell lines. And one criterium is to hypertriglyceridemia and or the hypofibrinogenemia. You can also find this and, a very helpful marker I think is the Ferritin. So the cut off that's given here is actually quite low. To put more, say if it's above 3,000, and this points towards HLH. And if it's above 10,000, HLH is probably very likely there. But these criteria are from children. So in other adults, the Ferritin often isn't that high. And another marker that's useful is sCD25, the soluble interlocking two

receptor if this is above a certain amount, it's also very suggestive of the disease. So these are some tests you can get in the laboratory more or less easy I would say. Another criterium is a decreased or absent NK cell activity. For this you need a more specialized lab. But it's something you can diagnose quickly, you need about two days. And the Hemophagocytosis, as mentioned in the first slide, is one of the criteria, but just be careful, it doesn't have to be there, especially not at the beginning. These diagnostic criteria were proposed in 1991. So they have been around for a long time. And originally, they were compiled in a retrospective analysis, and then they were revised again. But as I said before, it was defined in children. And you don't need to have all of these criteria; five has to be there to eight criteria, or you have to find one of the genes where it's known that it is associated with this HLH. The number of helpful findings that are not part of the published criteria, You almost always have transaminitis so the liver is almost always affected. So if you don't find any elevated transaminitis HLH is rather unlikely. You find an elevation of LDH. And one common complication of HLH is a CNS, an infection of the CNS. And in their lumbar puncture you can find pleocytosis, you can find some alterations in the MRI and neurological symptoms can be found in up to 70%. And sometimes in some cases, it can even be the oldest system. So, this is one of the most important slides in this presentation, I think. So I start with the take home message right at the beginning. And this is from a very good review Sheila Weitzman has written in 2011 and she said: "you can diagnose patients in the absence of the five criteria and some patients have five criteria but do not always have HLH." And I think this also is difficult. You have to see the entire clinical presentation, and you have to consider different aspects. And there's not a simple recipe to tell you this patient has HLH and this does not have HLH. So you need some experience and exchange with colleagues. Yeah, please send a message to us, if you have any questions or any suggestions. I think there are many different opinions on HLH and would also be interesting to see what you think. The pathophysiology of HLH is rather complicated. I just show you here are some of the main players and these are cytotoxic T-cells and NK cells and members of the representing cells like macrophages, monocytes and APCs. The reason why HLH is so detrimental is that you get an excessive immune activation, which leads so an excessive secretion of different cytokines and in the end organ damage. And the familial forms so that typically HLH usually resides some defects in the lymphocyte toxicity.

Dr Attarbaschi: Caroline may I interrupt you? Sorry?

Dr Hutter: Yeah, please?

Dr Attarbaschi: We have the first question in the chat, someone is asking what is the best way to diagnose isolate CNS, HLH?

Dr Hutter: Yeah, this is a nightmare a bit to diagnose it. I think, if you have a child that just has some high fever and some of the criteria, but just a few, in the end you have to do a number of punctures. Usually you find moderately elevated levels of protein and some pleocytosis.

Dr Attarbaschi: Thank you.

Dr Hutter: Okay? I told you the terminology is a bit difficult, because you'll say originally you have the primary HLH driven by mutations that have HLH as a main feature, meaning that all children have a mutation that leads to HLH. So they usually become ill in the first year of their life while a secondary HLH is driven by infection or malignancy et cetera. And then we have the MAS, which is HLH due to rheumatic disease or autoinflammation, and there is even some debate whether you should drop this name MAS and just say it is HLH, due to this rheumatic disease because it's a bit confusing, because at the end it's the same. And you can have the cytokine release syndrome, which is not HLH but can mimic HLH, and it can be due to different triggers, and also some CAR-T cells for example. So, if we had a classification that has been used for a long time and it is still being used by people, I think it's a bit helpful. As I said, the primary means that you have disruptive germline variants in genes that are important for perforin mediated cytotoxicity. And it's the HLH key disease manifestation and everybody gets it and usually in the first year of life. So that's at one end of the spectrum, which is here. And the other end of the spectrum would be that you have an adult patient who

gets a disease that fulfils clinical criteria for HLH, you don't find any of these genes that are usually mutated, but if you look a bit closer, you'll find a predisposition to immune dysregulation. And in real life, many patients are in between here. So you have to be careful. Even what you call a secondary HLH has features of the primary. So, many people dropped this name because it's the end, it's just one disease and different manifestations.

Dr Attarbaschi: Caroline someone in the chat is asking on infectious, infection driven HLH, and especially about COVID-19, but if I remember your presentation correctly, you will talk about this later on.

Dr Hutter: Yeah, I'll show a slide because there has been a bit in the literature so.

Dr Attarbaschi: Okay, good, then let's wait real...

Dr Hutter: I am quick, I think I'll do it sooner.

Dr Attarbaschi: It's fine.

Dr Hutter: Okay? Genetic defect vs predisposition. So I can say, you can see here a few genes that are associated this HLH. It's taken from a recent review by Scott Conna and Rebecca Marsh. I am not going through all of these genes, I just want to show you a general principle that is in the traditional familiar HLH, you'll have mutations in genes that lead to defective lymphocytes, granulocytes mediated cytotoxicity, and so, there is a Perforin gene in this aspect some other genes that are also important in this specific information. Then we have some pigmentary disorders that are also associated with HLH disorders syndromes like Chediak-Higashi syndrome, Hermansky-Puduk syndrome. Here the patients also have other symptoms. And because for example, also in the melanocytes, you have the melanosomes which are also basically a residual formation for the transfer of the pigments and so many of these patients, for example, have at least a partial albinism. And then we have the genes that are also associated with the histidine proliferative diseases. And they have important in infectious diseases triggered HLH. And also genes that are involved in inflammasome function, or CDC 42, which is a small Rho GTPase. That's important, usually for the actin cytoskeleton, but also can stimulate iron beta and iron 18 enhancing this inflammation. I just go into one gene, that's the Perforin because you can see the general principle of cytotoxicity and because Perforin was the first gene that was found to be associated with HLH. Also think it's also a radical system the body has developed to kill cell. So Perforin is a Por-forming protein and it permits cytotoxic proteases to enter the cytoplasm of and target cell and I want to show you here so that's the target cell, that's the cell that is cytotoxic lymphocyte wants to kill. So if you recognize this's target cell these vesicles are polarized and then they are released in this in this signups, it's like, this is the immunological signups and then in this vesicles, there's Granzyme B which is these proteins and Perforin. And this Perforin goes for the signups and then makes holes, punches holes in the target cells and allows the Granzyme B to enter the target cell and then leads to the cell death, of the cell, and the lymphocyte goes away and kills another cell. So that's the principle. And the first case of HLH regime that has been found was caused by this Perforin. So Perforin deficiency is quite an important reason why a patient develops HLH. If you think that somebody has this disease, how do you go on and I think of course it's not feasible to start sequencing right away, you have to wait at least for a week for research sometimes for many weeks. And what you should do is that, you do the normal legwork up that gives you some indication that this might be the case because of the mimics and because of the normal net variance. But you can do a flow analysis, that is already helpful because you can look at the pathway and expression in the flow and you can look at expression of other proteins that are involved in in these genes. So you already have an idea whether this gene might be mutated or not. Or you can do a CD107a mobilization, which helps you to measure the ability of lymphocytes and NK cells to kill her target cells. So you use initially a function essay to narrow down which genes might be affected. But of course I told you at the beginning, we now know that HLH genes expect on and that it might be more diverse than this. So, it's not just this impact lymphocyte cytotoxicity, but also the dysregulated inflammatory activity for example, or the impaired control of viruses. And in the center here, what is in red are the genes where most or all of the patients will develop HLH and

further on the outset of this, you can see genes where patients might develop the disease, but you need another trigger. So in the end, it all comes down to that HLH and, it develops because of a predisposition you have and because the immune system is challenged and then if there's a mistake in the immune system, it can't talk to such an urge and it leads to this hyper inflammation. And this is what I think this graph quite nicely depicts. Here, you have hyperinflammation because of a lupus for example, because of this rheumatoid disease, which is something else that comes, and it can develop an HLH disease, and the same as with certain viruses, like EBV, for example. So if you have many genes that are helpful to do just this. Look at the familial genes, It depends a bit on the age of the patient and the history. This is a diagnostic algorithm that comes from colleagues in Texas. They have published what they think people should do. I like this graph a lot because it says that this is a unhappy patient and it says: "consider HLH." This is something you should bear in mind. If someone is very sick, we always have to think could it be HLH? So what this suggests is that patients, if they are positive for this diagnostic criteria you do these factors SS as I've shown you before, if they're abnormal, we do a targeted sequencing because these functions SS as already tell you which genes might be affected. However, if these SS are normal, but the patient needs therapy because the patient is quite ill, you have to do a bit more extensive genetic analysis and you will do a whole genome sequencing or whole exome sequencing or RNA sequencing. So this is a stepwise approach you can use which is a bit helpful, I think. The only thing I disagree a bit was this year where they say observe, even if a patient as I told you before, it's not positive for all of these criteria, if the patient is sick, go on and proceed with this analysis because he might still have HLH. Again, this diagnostic algorithm was developed by paediatricians. So in adults it's a bit different. And there's some debate whether a German testing makes any sense or not. And there's another indication of how familial are primary and secondary HLH has to be seen a bit critically. And one thing that was mentioned is that it's not easy to define the boundary between positive genetic alterations and the genetic variant in adults. So for example, if you find a mutation in Perforin, which is not deleterious, but just leads to a bit of a lower Perforin function, the patient might eventually develop HLH but not at 100%. It's an add on for example, and this is a nice review, I suggest that you read it. Maybe HLH would be seen rather like a Pollock picture and not like a picture of a Mondrian, which is quite linear. HLH is very difficult in the sense of understanding how it develops. So now, is it important at all that I know whether it's primary or secondary? and if all data suggests no, it doesn't matter. Because if you look at the last published large patient series, the results of the HLH 2004 study, which was a paediatric study, it didn't matter. So, whether the patients had primary familial HLH or not, this variable is about the same. So in the acute setting, it's not at all head from the acute setting you just have, does the patient fulfils the criteria to think it could be HLH? and if I say yes, then you have to start treating the patient. I think that's perhaps the most important message to you. You can have different immune challenges. So if you think a patient might have HLH, you have to look for all these different triggers. And some of these triggers for example, is autoimmune diseases, where I said we call it MAS if a patient with autoimmune disease develops HLH and here we have the lupus and rheumatic disease with this as the main reasons for this. And it's very difficult also at the beginning to say is this HLH or is this MAS? Does this patient have a rheumatoid disease because the HLH could also be the first manifestation? And another important trigger is malignancy, that's more important in adults than in children so in adults it occurs in 45% of cases. It resides from sides of integration by the malignant cells and antigen stimulation. And depending on the cancer, the prevalence can be quite high up to 20%. So what do you do if the patient has cancer? Should you know malignancy directed regimen or should you treat HLH? I think this is perhaps something to knows more about it than I do. But I would say it must be decided case-by-case. You cannot always tell but usually it is important to treat the underlying disease rather than to try to try to treat the HLH symptoms.

Dr Attarbaschi: I can comment very shortly in children and adolescents Non-Hodgkin's lymphoma, especially anaplastic large cell lymphoma or subcutaneous panniculitis like T-cell lymphomas have a risk for secondary malignancy associated HLH. In these both disorders we have the advantage that malignancy directed therapy is already including steroids or it may be enough to start with malignancy directed therapy and by the steroids

you also treat secondary HLH, but we have also seen HLH in acute myeloid leukaemia patients, for example, in monocytic an acute monocytic or monoblastic leukaemia. And here our therapies do not include steroids and the question to you Caroline would be would you add steroids to malignancy directed chemotherapy? If it is not included in the chemotherapy? Or would you leave some time? One or two days to see whether the chemotherapy works in also reducing the HLH in these patients?

Dr Hutter: I think it really depends on the case, but I would like to add the steroids, I think.

Dr Attarbaschi: it's the same with me, I would also.

Dr Hutter: We like steroids.

Dr Attarbaschi: Okay, thank you, thank you.

Dr Hutter: Yeah. So those two slides are rather off topic on CAR-T cells, I think because we are very interested in CAR T-cells, especially on T-cells. And we know that HLH is observed in a low percentage of patients who receive CAR- T-cells and the diagnosis is, of course, super difficult because all patients have some sort of cytokine release syndrome. And so how do you discern this from HLH in the first instance and also you don't, you really don't want to treat it because you want then Car-T to work. So it might kill you if you start the steroids for example, in this patience, but one has to be careful because there have been fatalities because of the fulminant inflammation, and suggestive of HLH in that context would be very high Ferritin levels and a great three toxicity. And their colleagues in this review, in Nature reviews in oncology they suggest that you should suspect an HLH. In this context, you should still manage anti-IL6 we should manage a normal CRS that needs treatment in Car-T cell, Patients need to be put on corticosteroids and only if there's no response after 48 hours, consider adding treatment for refractory HLH. But I think everybody would be extremely unhappy doing this. Because I suppose it should it means that you can't forget this CAR-T-cell therapy, right?

Dr Attarbaschi: Yeah, but as you said HLH, it is rare in CAR-T-cell therapies, but it can be very, very dangerous, life threatening and fatalities have been reported. And I think, the CART-T-cell experts at the moment are learning that giving steroids earlier on in tweeting whatever CRS crest or MAS, is not so dangerous in killing the CAR-T-cells and people I think are getting more generous, I think writing English word in starting steroids early on, especially in a situation where HLH it can be life threatening and that's why you should not withhold the steroids because they think you will destroy the CAR-T-cells.

Dr Hutter: Okay, so now we come to the SARS-COVID. I have one slide because, it has been in most newspapers that, people have been asking that there's a subgroup of COVID-19 patients that have a cytokine storm syndrome. And it seems to correlate with disease severity, there was this widely written article in Lancet in March. And so the question, of course, is from many people who do not start treating the hyperinflammation you see, for example, that couple of clinical trials that use interleukin six and the interlocking one inhibitors in the settings. I have to say I can't comment on this because I think the verdict is still out. One has to be extremely careful in just doing a symptomatic treatment, if the immune system is trying to control the virus, so perhaps it adds a little map, but it's not helpful. But I don't know I think probably it also depends on each patient. But I want to point out it is an extremely interesting paper that came out recently that showed that variations in Perforin in calcium function may be one of the factors that decide whether a patient becomes very ill or not. And that's interesting because I think everyone wants to find out what are the risk factors in developing a more severe disease and here in orange you can see risk factors like age, gender, height, body mass index, age over 70 years. And this all leads to reduce cytotoxic cells and Perforin functionality. So, of course, this is just a correlation, it doesn't say it's a causation, but what it has been described is that, for example, this is a sudden mutation in Perforin that this leads to a more severe COVID-19 disease, than in vital Perforin in patients. So, there seems to be some predisposition, and you seem to have a lower disease severity, if you have optimal cytotoxic function, which, for example, seems to behave for certain therapies that stimulates cytotoxic cells and Perforin, like Metformin, for example, or also by

vaccinations that boost NK cell activity. I think you have all heard about the data on the tuberculosis vaccination, that it seems to protect from a more severe disease. So this is what we can say. But I think before we have to first resides of large studies that are very controlled. It's difficult to say whether we should interrupt this cytokine storm syndrome early or not in the setting of the infection. So, SARS-CoV-2 variables. So the treatment. One thing is you have to suppress well inflammation quickly, you have to be really fast. You cannot wait in many instances, especially in small children who are very sick. There is a protocol that is 94 protocol that Histiocyte Society, which has improved the survival of 25 to around 55%. And the survival rate after the stem cell transplantation is currently even between 80 and 90%. So standard of care still is at least in paediatrics. The induction therapy that is based on a study that was published in 1994, which uses at topocyte and dexamethasone to bring these children into remission that enables them to either have a maintenance therapy or if you think that they have a genetic predisposition to do a transplant. So what you should always do is diagnose HLH or already start HLA, genotyping and look for potential donor in case the patient needs stem cell transplant. Current data posted at the beginning is giving twice a week. Even if the patient is cytopenic and has a bad liver function, it doesn't matter. The patient needs the treatment otherwise he will not get better and the dexamethasone which is tapered every two weeks. I don't go into detail in this slide, I just want to point out that this is the HLH 1994 treatment, which is the basis for us paediatricians. It is based on this primary HLH which is based on the problem that's after toxicity, but the more we learn about HLH and the different reasons like inflammasome homeostasis might be impaired or the impaired answer on viral infections. The more we can use perhaps different drugs in different settings and just show you some of them. One very prominent, one that might even be become a frontline treatment choice is the anti-interferon gamma antibody. It's now called Gamifant. it's already FDA approved. And there is a very recent paper in the New England Journal where they just published on 34 patients, and not a lot. But there is also research and they're very promising and it's not toxic. So I think this is an excellent choice or maybe an excellent choice in combination for many patients. The CAMPATH has been used for a long time. In settings with also an HLH, there's some experience and there are a number of clinical trials currently testing these again, on larger patient numbers. So far, they're not very high patient numbers reported and other treatment approaches, which I think are also interesting are that Anakinra, it's an interlocking and receptor antagonist. You would more use this for the MAS so for patients who have an underlying rheumatologic disease whether HLH at the beginning often is not that aggressive. There are some reports on Ruxolitinib, a JAK-inhibitor. There are not many trials open that test Ruxolitinib in HLH, and there is very interesting data in a mouse model. And the Tandekinig-alfa is an interleukin-18 inhibitor, and that's, for example, interesting in, diseases triggered by an inflammasome activation. As I said before, the stem cell transplant is the only course of treatment for patients that are at risk for recurrent episodes of HLH. So usually, the small infant that has a cytotoxic T-cell, this function and that different conditioning regimens, we could probably talk a lot about this in itself. But all in all, reduce toxicity scheme seems to be much better than the milder ablative conditioning regiments, and you don't need an extreme high documentary, some 20 to 30% seems to protect against reactivation. But if there are no defects in lymphocyte cytotoxicity, you could try a less aggressive treatment strategy like at the beginning just take some Metasone and Anakinra in these patients. So to summarize HLH is not a single disease, but as a syndrome due to severe hyperinflammatory, uncontrolled reaction. It triggers induce hyperketonaemia, and patients with HLH have inborn immune deficiency or an acquired or suspected inability to cope with certain triggers and their characters to finding in genetic forms is this decreased function of NK and T-lymphocytes. And my take home message for you is, it's life threatening, it can be life threatening. You can at the beginning just think it's a sepsis and that's it is just a bit too slow and reacting. But you should always consider it can mimic different diseases. But just stay alert and just inspect it. And don't wait until five of the eight criteria are fulfilled because this might happen too late.

Dr Attarbaschi: Thank you very much Caroline for this very nice and great presentation as always. Before I ask my two questions that are left, there's one question in the chat. Someone is asking whether there is any association with Kawasaki syndrome and HLH.

Dr Hutter: Yeah, there is actually. So the bonus that often, so Kawasaki syndrome predisposes to HLH. So you can on the basis of a Kawasaki syndrome, you can develop a full blown HLH, which you also need to treat and not just for the Kawasaki syndrome, but also for an HLH. And also, there are a few reports that say that often patients with HLH together had Kawasaki syndrome, had the clinical presentation, that at the beginning made the doctor think that his patient has a Kawasaki syndrome. I remember myself a patient we had in our hospital a couple of years ago where we thought she had a Kawasaki syndrome, at the beginning she didn't have this analysis, but otherwise it looked like it, but it was not.

Dr Attarbaschi: Thank you. Two further questions in the chat concerning therapy elements that you did not mention one is cyclosporine A, and the second one is IV immunoglobulins.

Dr Hutter: Yeah.

Dr Attarbaschi: Okay. In case of virus triggered HLH.

Dr Hutter: Actually I should have mentioned them. So, the CSA is still mainstay in the standard of care induction therapy. You add this a bit later in the 1994 then in the 2004 study, but it should be added to the therapy and immunoglobulins, should always be considered in the in the viral tracing HLH. So, for example, if a patient has, I didn't talk, about the triggered HLH but there you would give immunoglobulins and of course cytotoxins. So everything that tries to get the virus in check should be used.

Dr Attarbaschi: There's another question in the chat concerning, I think if I understand it correctly, the cytokine composition in COVID-19 patients and classical HLH let's say is there any difference already known?

Dr Hutter: I don't know of a difference and all that impose you'll find high levels of interleukin 10 and six and one. But whether, for example, interleukin six is a bit lower in HLH than in COVID-19 triggered disease. I don't know.

Dr Attarbaschi: I think we need some more time to answer questions on COVID-19 with HLH and many other questions. I have one question. If you have a child for example, with familial HLH, who has been successfully transplanted, what are the current recommendations if the child gets a sibling in which the mutation is also detected? Are you recommending at the moment to do some prophylactic transplantation or not?

Dr Hutter: If it's one of these genes where we know the patient will develop HLH, for sure, like the Perforin mutations that we know then we do this because prophylactically now.

Dr Attarbaschi: So you would go for a transplant in the neonatal period because you will not wait.

Dr Hutter: Yeah.

Dr Attarbaschi: Okay?

Dr Hutter: I think we have done this plenty. I mean, remember the child who developed HLH in utero?

Dr Attarbaschi: Yeah.

Dr Hutter: But we were too late.

Dr Attarbaschi: Yeah. Okay, so let's see whether there are still questions left in the chat? I think not. So I think that we can really finish on time. So if there are no further questions. I would like to thank you, to you for taking this invitation to give this talk on this very specific disease. But I think it's important to know about this disease because as you have very nicely shown, it's almost always life threatening, and you have to treat this disease immediately. So thank you very much. And I hope that people who are listening to us learned a lot and now know how to treat HLH exactly. Ans thank you very much Caroline

Dr Hutter: Thanks Andishe and thanks for attending.

Dr Attarbaschi: So we see each other tomorrow and all the best to all the people who listen to our discussion. Thank you very much.

Dr Hutter: Bye.