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Therapy-related complications: Differentiation syndrome after retinoid application, and secondary HLH

Dr James: Lovely, thank you. So, as the lady has just said, I'm Dr Beki James, and I am a paediatric haematologist in Leeds Children's Hospital. And my colleague, Dr Bob Phillips, is on the other line.

Dr Phillips: Hello, I'm Bob, I'm a paediatric oncologist in Leeds Children's Hospital as well. And it would be really lovely if you do want to ask any questions through this presentation to pop them in the Q&A, which if you sort of wiggle your mouse around, is down at the bottom, near the participants. And if you could use the Q&A rather than the chat, that would be probably the best. But if you hit the wrong button, don't worry, we'll try and catch them from all of those areas. I think, Beki is... Yes, there is Beki, Beki's pen. I will shut up and hand over to Dr James.

Dr James: Brilliant, so this evening, we're going to have a bit of a run through two things: differentiation syndrome and secondary HLH. Now, we're going to start off by just thinking about this *Primum non nocere*. Now, you will have noticed that in medicine, we try whenever possible to speak a different language. So, here we're speaking Latin obviously, and this is from Hippocrates. It's not from the Hippocratic Oath, it's from another of his works called "Epidemics," which seems very topical at the moment. It's actually called, "Of the Epidemics". And this is here because it's really, really essential whenever we use a drug that we know what harm it might do, and that we're ready to pre-empt that.

So, if we now think about APL, we can think about differentiation syndrome which is seen most often in APL. So, what is APL? Well, you have a blast here. You see them in the bone marrow usually. You might see them in the blood if you've got leukaemia. But this is the blast here. And then, remember, down the microscope what you see is this becomes a promyelocyte, the very big nucleus again with these nucleoli like this here. And then, what you have is granules now coming around the outside, then, this becomes a little bit smaller. The nucleus is now becoming a bit more manageable, the nucleoli aren't so evident, and you're getting more of these lovely azurophilic or blue-loving granules. And then, the nucleus now is becoming like this. And now, we've gone from promyelocyte to monocyte to a metamyelocyte. This is perhaps even becoming a band form. Beginning now to look much more like a neutrophil. And then, it reaches its destination which is to become the kind of neutrophil that we all welcome in the blood because they're so good at fighting bacteria, being anti-inflammatory and healing up anything. So, this differentiation happens normally in a healthy way in the bone marrow. And when they're at this stage, the neutrophil is sent out into the blood to help. If you have a stressed bone marrow, they might be sent out a bit earlier. So, differentiation is normal, but differentiation syndrome is what happens in APL when you give ATRA and arsenic. You give these and you get this accelerated moving through that actually causes problems.

Now, APL: we should just pause and say it is a medical emergency. When I was preparing the talk, one of the things that I found written down was that the *diagnosis* of APL was a medical emergency. Actually, it's before that. It's as soon as you *think* you might have a patient with APL that you need to be jumping up and down. And I cannot stress this enough because APL is a condition with a 98% to almost a 100% survival if treated rapidly. But most of us, by the time we've got to my stage of the career have sadly seen children, young people or whatever age patients we look after who have actually died from acute bleeds in the first few days of having APL. And we want to try and stop that happening. So, as soon as you get called about a pancytopenic patient, ask yourself *every time*, could this be APL? And if there's a chance it could be APL, get yourself to the lab, or find a colleague to go to the lab, and look down the microscope. Don't wait for the fancy tests. Look down the microscope. If you think it looks like it might be APL, it is better to get the ATRA in at that point and give a dose of ATRA and then, stop it because it's not APL than to hang on for eight hours because you're waiting for diagnosis. So, as soon as you suspect it, you need to have a phone in your right hand and a phone in your left. With your right hand you are phoning down to your Blood Bank to say, "I'm going to need platelets, cryoprecipitate, and FFP for my patient". With your left hand, you're phoning the pharmacy to say "I'm going to need ATRA". It is really, really important. And that is because it is potentially fatal.

Now, not only is APL potentially fatal, but differentiation syndrome, referred to here as DS, is also potentially fatal. So, you've got a really high-risk situation here and actually, differentiation syndrome is therefore associated with a reduced overall survival. It's also associated, interestingly, with a reduced event-free survival. Now, we don't know if that's because you have a group who are more likely to have differentiation syndrome who then do worse because they were always going to do worse because the characteristics of those who are at risk of both things overlap. Or, whether because of the differentiation syndrome, people don't get the same treatment, and so those people then end up being more likely to relapse.

So, what is the incidence? Well, the incidence is not clear, and it's set at about 2% to 48% in various papers. Well, that is a big gap as you'll spot. That is actually between 1 and 50 to about 50%! And the reason why it's so different is because in the papers what you have is different protocols for treating the APL. In addition, you have different strategies for managing the differentiation syndrome, and you also have different diagnostic criteria.

But what actually is going on? What is the pathogenesis of APL? Well, three things are happening. Remember we're going from the promyelocyte stage and we are really, really rapidly, making it become a neutrophil, so, it's zooming through to this neutrophil stage here. And all kinds of problems ensue. The three main problems, we're going to think about in the pathogenesis are:

1. first, you get cytokine release. Cytokines, remember, are little protein-based molecules that are membrane-bound. Actually, in my mind they're always orange. I'm going to put them here as orange. And they signal. I think they're a bit like, you know in old wartime films, you get the little operators on the radios going, "Beep beep, beep, beep beep, beep," sending out messages. That's how I imagine these little cytokines, sending out their messages and signalling different pathways like that. They signal mostly in the immune response, and they can cause an overactive situation. The ones responsible here are IL-1, IL-6, IL-8, and TNF-alpha.

2. the second thing that happens is you get release of something called Cathepsin G. Now Cathepsin G is really, really exciting because when we see these little blue azurophilic granules, one of the things they actually contain is the Cathepsin G. And so, you can see what's happening here is you're getting all of these bursting and releasing their contents, and so, you're going to get release of Cathepsin G. And I'll tell you in a minute what that actually does.

3. the third thing that happens is that you get an increase in adhesion molecules. So, adhesion increases or simply put, they just become sticky. And this is because of three particular products. The first one is lymphocyte associated functional antigen (LAF-1). Got my numbers right. The second one is intracellular adhesion molecule 2, (ICAM-2). And the third one is my favourite, which is the very late antigen 4 (VLA4), which is a great name for something.

So, what happens because of these is you get two main pathways happening: you get increased vascular permeability - you become leaky; and, you get direct endothelial damage, especially with the Cathepsin G. So, because of this what you're going to see is weight gain. You're going to see pleural and pericardial effusions. (That's a heart for those of you who are looking at the drawing). You're going to get pulmonary oedema because not only do you get fluid *between* the lungs and the lining or *between* the pericardium and the heart, you're going to get the actual organs *themselves* becoming soggy. Now, as well as them become soggy with fluid, you also get organ infiltration with the white cells because they're very sticky and they're escaping between the endothelial cells which are now not joined up tightly to another, so, you get organ infiltration. And that's a particular problem in the lungs and in the kidneys, potentially in the brain. So, organ infiltration by the white blood cells. You also get reduced organ perfusion because you've got all this stuff blocking the normal flow of blood vessels, so, diffused organ perfusion. Now you can see that you're going to be running into organ failure. And you can imagine that somebody like this is actually very sick and they'll often now be going into a shock-type picture. You also get some other cytokine side effects. These will cause some things like fever. You'll get a tachycardia going on.

So, there are lots of different things happening to the patient. And so, it's from these effects, this pathogenesis that you can see here with these two - vascular permeability and endothelial damage - that we generate our diagnostic criteria. So, I can put over here the diagnostic criteria. Well, I mentioned at the start that one of the reasons why we got such a variable incidence is because there's very varied diagnostic criteria. But I think increasingly there's a consensus forming around the ones from the Spanish PETHEMA group and this lovely paper in 2009 by Montesinos. And you'll hear some people who refer to the Montesinos criteria. And that's from this paper, it's in Blood for those of you who want to look that up. So, if you think back to the pathogenesis, it helps makes sense of what the criteria are.

So, there's seven criteria:

1. the first one, we said that you might get a fever, so a fever greater or equal to 38
2. The second is that you get a 5kg weight gain, which actually is hopeless in children because 5kg might be half their weight. So, in children we tend to think of it in percentages as 7% weight gain.
3. you get hypotension
4. you get an increased respiratory rate
5. on a chest X-ray you'll get opacities. Really, really important you do the chest x-ray. And it may be that you did one when the patient came in. But when they deteriorate, you absolutely need to do another one, even if it's the middle of the night.
6. clinically, you may detect a pleural effusion that counts for the same point as cardiac effusion. Again, repeat the examination, repeat the echo if you have done one but their condition changes.
7. they may have an acute kidney injury, which for these purposes counts as any creatinine about 123 or above the normal range.

So, how many of these criteria do you actually need? Well, it's very generous. So, you only actually need one criterion and a clinical suspicion - this is enough to make a diagnosis of differentiation syndrome. If you have three at least, it's moderate differentiation syndrome. And if you have four at least, it is severe differentiation syndrome. And severe absolutely means you should stop what you're doing and attend to this patient. Really, you should in moderate and really should even if it's just starting because this is only

going to progress from where it's very, very unlikely to turn around absent some support. And as I say, it can be rapidly fatal.

Now, there are a few other things that are described. In fact, if you go to PubMed and look up differentiation syndrome, you'll find lots of different things mentioned. But practically, although it's not part of the criteria, patient will often complain of myalgia. Diffuse alveola haemorrhage is mentioned. And remember bleeding is a very, very big problem with the APL patients altogether. Myocarditis has been described. People have also described things like Sweet Syndrome of the skin (neutrophilic dermatosis syndrome) and you can get CNS problems. But if you're getting CNS problems, you're probably a bit too late with the whole thing. Doesn't mean you shouldn't try.

So, what do you do when you've got somebody in this situation? The first thing is to be confident about your diagnosis. Because the treatment is unlikely to cause harm but it's very, very likely to turn everything around. The treatment consists, first of all, of steroids. And we use dexamethasone. As I say, in children we tend to think very much in mg/kg or m^3, so we'd be giving 5mg/m^3. We cap at 10 mg and mostly for adult patients it would just be 10 mg BD. And using this has decreased mortality from about 10% to 1%, so, a tenfold improvement. Reassess after 24 hours and see how your patient is doing. If they are not better then, double that, then go to QDS, or just double the dose, because you really, really need to get on top of this. And you can taper once you've got complete resolution.

So, that's the first thing: getting on with steroids. And that's why it doesn't matter if you're thinking, "Oh, I'm not quite sure, hmmm, they've got two criteria but it could be this, it could be that." Crack on. But yes, absolutely be thinking about your differential diagnosis because we need to make sure we're not missing something else. The reason why it looks like shock may be because, actually, they have got septic shock. And make sure you're thinking and covering for all those infectious things. Is it septic shock? Do your cultures, give them antibiotics, protect them. Might it be a PE? Differentiation syndrome is pro-coagulant. Remember, we've just said that if you have APL you might bleed. But actually, if you have differentiation syndrome, it's actually pro-coagulant. We do see patients with PEs. That might be why they have gone into oxygen, why they are tachypnoeic. Or, have they dropped their blood pressure and become tachycardic because they've had a bleed? Because we know they are very prone to bleeding. Have they developed effusions because they've gone into heart failure? Have they got anaphylaxis, because, some of the medicines that we use in the treatment can cause anaphylaxis? Or have they got transfusion-associated circulatory overload because of the different products they've had to keep them safe from their bleeding problems?

So, that brings us really to supportive care. So alongside this, you've got really, really great supportive care. Don't be so focused on just giving steroids that you forget the wraparound eg. oxygen; early ICU referral - because some of these patients will need ventilation; furosemide to get the fluid off. Particularly important for people who look after children to learn because often we're a lot more wary of giving them furosemide than our adult colleagues. And manage the coagulopathy. Sometimes in the first few days you'll be pouring blood products into these patients, but that can be lifesaving. So, the parameters that I would suggest to you, that you need to bear in mind are: keeping the platelet count above 50 $x10^9$/l with platelets; a normal APTT, and for that we use FFP; and, you want to have a Clauss fibrinogen (ie a direct measure of fibrinogen, not just a calculated one on the coagulation screen because some hospitals just give a calculated version) but a direct fibrinogen, that is > 1 to 1.5, and we use cryoprecipitate to do that. And I would still recommend cryoprecipitate because of this pro-coagulant feature. It is a bit more tried and tested than using fibrinogen concentrate in this situation.

You also need to remember your actual leukaemia management, because it's the leukaemia that's really causing the problem. So, what about the ATRA or the ATO (the arsenic)? Well, in most situations you want to continue because what we want to do, is to get the blast cells differentiated and out of the system. And

they are all being sent out of the bone marrow. So, you continue, it if possible. But, you may have to stop. And we stop it if someone has got severe - so that was, remember, four or more criteria. We stop it if they've got organ failure. If someone is going on to dialysis or is going to be ventilated, we stop it. If they are in ICU, or PICU (the paediatric equivalent) we will stop it. But, you want to be restarting it as soon as you can. So, restart when they are no longer severe. Restart when possible, because you've got to keep on treating the disease.

Now, something else you could do for the disease is actually to give the chemo. So, if you're planning to give them idarubicin, for example, because they were high-risk, it looks as if it is better to give that because, actually, the incidence of differentiation syndrome if you just give arsenic and ATRA is about 50%. If you give anthracycline, it looks as if you halve that. And that's probably because what you're actually doing is killing off the cells and you are seeing far less of an ongoing release of azurophilic granules and cytokine and cathepsin release than you would otherwise. And so, that might be either idarubicin or it might be hydroxycarbamide. We don't give the low-risk patient with APL anthracycline. But if the white cell count is between 10 to 50 $x10^9$/l and they've got differentiation syndrome, then it is recommended to give hydroxycarbamide (also called hydroxyurea) because what you are doing is just killing their cells and getting them out of the system. And then when it is below 10, you can stop again.

So, that has been a sort of whistle stop tour really of differentiation syndrome. I'm just going to pause there and see if anybody has any questions about that before we move on to secondary HLH.

Dr Phillips: Thank you, Beki, that was fascinating stuff. As a reminder, if you've got questions, please do put them in that question-and-answer box at the bottom. If you miss and you hit the chat, that's okay too. We can cope with that. Is there anybody that wants to ask a question or type one in quickly now? There will be an opportunity at the end. It's just, are you so tense with a question that your brain couldn't cope with moving onto the next section without asking it? Feel free to throw that question out now-*ish* for the first time of asking, and the second time of asking, and that's it. The opportunity to ask mid-session questions live and have them answered is about to expire as Beki's taking a breath in, and is on to Part 2 of things you didn't realise you didn't know about the shape of wiggly things under the microscope.

Dr James: Great, so let's think about HLH and the management of secondary HLH. And this is really important because people have sometimes in the past thought secondary HLH is less important than primary HLH, and less severe, but actually it can be very severe in many cases. I am going to suggest to you a way of thinking about HLH and its categorization, which enable us to think about the treatment.

So, with primary HLH, you have your familial types. These are the very classic perforin mutations, mutations in UNC13D, ie familial HLH types 1 to 5, which, whilst very interesting, are not the purpose of this talk. And then you have your other genetic types of HLH. Familial HLH tends to be part of a very discrete syndrome where the main problem is HLH. The other genetic forms are paediatric immune deficiencies where they are often part of much bigger syndromes. You are thinking about things here like Chediak-Higashi or Griscelli, maybe DiGeorge, or XIAP. And these are part of a syndrome that is likely to be causing a primary immunodeficiency.

Now, you also get secondary HLH. So, secondary HLH has a number of different causes and I'm just going to pause a second while you, in your mind, think, "What is she about to write down?" It is really good to keep your brains alert.

So, the first thing I'm going to write down is infection. Now, any infection can cause HLH, but we think particularly about viruses. One of the most common viruses that causes it is EBV, adenovirus is another. And this is where you see a bit of interesting stuff going on because actually then you've got a bit of overlap here with the XIAP and the EBV group. TB is a relatively common cause. But really, if any infection can trigger a hyper-inflammatory response then that is the hallmark of HLH.

We think about autoimmune disorders, and you come over here for that. So, autoimmune disorders. Now, it used to be that people referred to macrophage activation syndrome. Macrophage activation syndrome is HLH. Just another name for it and we now understand that. Interestingly, in the circular loop here, macrophage activation syndrome was the phrase coined in 1985 by Claude Griscelli. But now we understand that actually it is the same process as now we know some more about it, so we have stopped calling it something different.

The next thing we are going to think about here is malignancy. And it is particularly the lympho-proliferative disorders. Why? Because they are the ones that particularly cause an acquired immunodeficiency in the way that is important for HLH. Also, you have people who've had a hematopoietic stem-cell transplant. They also, obviously, have an acquired immune-deficiency. And then, you have drugs. Drugs are always such a problem. Now, you have rare causes. And that might be something like lamotrigine. Always, always, scrutinise drugs and ask what someone might have had if you have someone presenting.

And then you have absolutely classic therapies like CAR T cells where we're *expecting* that they might do this. And in CAR T cells we think about the cytokine release syndrome of which HLH is one of the manifestations. And that's the way we are currently thinking about it. And maybe, in 2 years' time, when we talk, we will be saying, "oh, yes, people used to talk about CRS, but actually, now we know it is always HLH and the whole thing is just a spectrum". But anyway, I am not talking about CAR T cell HLH here, because it is very well understood and we give tocilizumab. We may or may not give steroids - we give them if somebody is severe or not responding. And as you would expect, your likelihood of getting HLH after CAR T cells relates directly to having a high tumour burden or having a high CAR T cell dose, which is just logical because you have got more of army A or more of the opposing army B. And also, it varies with the CAR T cell type that you're having, but it doesn't seem to be impacted by what you have had beforehand.

So, for HLH, if we just recap briefly, we normally use the diagnostic criteria from HLH-2004. And you have to have 5 of 8 of these:

1. fever
2. big spleen
3. cytopenias
4. high ferritin - and in secondary HLH, as in primary HLH, these two are particularly important. Your platelet count and ferritin are two of the most rapid indications that you will get as to whether you are responding. Another thing that you will see as a very rapid marker of response, is lactate, although it is not one of the actual criteria for having HLH.
5. low fibrinogen, or high triglycerides gets you the same point
6. HLH on biopsy
7. high soluble CD25 (I put that down there because often that takes a lot longer)
8. natural killer dysfunction on assays.

Actually, my experience is that in secondary HLH you very often do not see bone marrow biopsy features of HLH necessarily. And then in adults, we also have something called the H-score, which you can type into a computer. It is actually based on 9 variables and they are weighted differently. And it comes up with a score that tells you how likely a patient is to have HLH. But, unfortunately for paediatrics, it is based on adults, and adult HLH is very skewed towards malignancy, which is different to paediatrics. We are not sure if it is generalizable for the paediatric population, but it can be a useful way, again, of backing up your suspicion.

So, what is HLH? We thought about, remember, how differentiation syndrome was a consequence of the increased vascular permeability in foetal damage. And it's very similar here. What you have is you have

impaired cell killing. The cell has recognised that you have an infection. It is shouting out, "Kill me, kill me, kill me. I've got the invaders inside me," but your body just cannot do that. And so, it shouts louder and louder and louder. And so, you get this hyper inflammatory response. And then that leads to a whole cytokine storm. So, not entirely dissimilar at all from differentiation syndrome that we were thinking about. Now when children present to us, they don't present at this end of the sheet. They actually present kind of over here with “I might have HLH”. They don't tell us at the start, usually, what they have got. So, my schema for thinking about how I treat them is based actually on real life. There is no point in me going through each one of those and telling you how you can treat it because you don't know at the start which one they have got.

So, at the start you have somebody with a diagnosis of HLH. Now, I would say that sometimes you have a “very, very likely or possibly even definite HLH”. When I met the younger sibling of a patient of mine who had presented with HLH, and the younger sibling now had the same features, they were in this group. When I came back from leave at Christmas one year and found that the cousin of a patient of mine who had had HLH had presented with pancytopenia, fevers, large spleen, they were in this group. So, some patients are in this group. Some patients present with a “possible primary but it could be a secondary”. And some patients present with a “clear secondary HLH”.

Let's think about those three groups and think about how we would manage each of those in turn. My talk is about secondary, but it is important to think about this because as I say we don't know when we are first seeing them which it is. So, first the very, very likely ones. Bit of history here. We started off in 1994 with HLH 1994. And in that we gave etoposide (etoposide is actually from a root, it derives from the mandrake root), and we gave dexamethasone. And then, after you got past induction, you added in cyclosporin. We had about a 55% overall survival from that, which doesn't sound very good, but actually was a lot, lot better than anything we had had before that. There was a suggestion that bringing the ciclosporin forward into induction would help, so, in the 2004 protocol there were the criteria established for diagnosing that we just talked about. We gave the dexamethasone and we gave cyclosporin here induction. But actually, we saw roughly a similar overall survival, although, interestingly, we saw more actually get to transplant. It may be that actually transplants were the problem here rather than ciclosporin. Remember in addition to those three good things, we're giving IVIG. IVIG is helpful very often for all kinds of reasons.

But really there is a bit of concern here, because if we are honest with ourselves, this isn't great. There is a lot of reticence from my colleagues, particularly outside of oncology about giving etoposide. There I a lot of concern from people about giving cyclosporin to people. So, because of this there has been a new regimen that's come up from the Necker Institute, with brilliant work done by Despina Mouschos and her team substituting the etoposide with alemtuzumab. And again, you give methyl prednisolone (methyl prednisolone is more anti-inflammatory than dexamethasone) and cyclosporin and gett them to transplant because this is the group that you want to get to transplant. The results from their work show over 90% overall survival, much better than anything else we can use so far.

But we don't give alemtuzumab to the secondaries. We only give it in this group here. Now, I am descriving our institutional practice. Actually, there are lots and lots of ways of practising here. What about if you have somebody with a *possible* primary? Well, in this group I would go to our standard HLH-2004 because you may discover there's something else going on that means you don't want to give them Campath. And particularly, out of interest, if you have EBV, we think that Campath might be adverse.

The next group is those who have a clear secondary diagnosis. If you have a malignancy, for example, you might have somebody who has a B-ALL, a lymphoproliferative disorder, and then they develop HLH - and we probably see this in the Children's Hospital here about once every two years - I would also be giving as my first line HLH-2004, because it is well tried and tested and you can very often peel back very quickly. They often tend to go into remission provided you get in there quickly.

Your autoimmune group, your infection triggered HLH, and also maybe the HLH occurring after transplant. I don't really want to be giving these people etoposide. And actually, for these guys the best data I think is now on anakinra. Anakinra, if you remember, is an IL-1 blocker (IL-1 alpha and beta blocker). It was actually developed in sepsis, for use in sepsis, so, it's something that we are much more comfortable with using in an infection. It is also used in maintenance for children with rheumatoid arthritis, JIA. So, again, we are much more comfortable, and our colleagues are, in using it in this situation. It is usually in the hospital, which is a great bonus, and we are do not have to apply for it especially, it is usually relatively easy to give. You can give it intravenously (IV) or subcutaneously. We very often have to give it IV because the patients are often very oedematous, and you tend to see a response actually quite rapidly. In 24 to 40 hours, you might be seeing a response. You give it a dose of 2mg/kg and you can escalate quite rapidly, and then there is a dose cap. Alongside this you are giving methyl pred. You may be giving cyclosporin and you are giving IVIG. Again, if a child is not very sick, you might just hold off cyclosporine and see whether you can turn it around with anakinra, methyl pred and IVIG alone.

And if I wasn't winning with the first group, I would then move to Anakinra in my sort of mental map. Alongside all these things you have got good supportive care ie the anti-infection measures. And you have got looking for your differential diagnosis. The tests that you are doing are also geared to thinking, to trying to answer the question “is it primary or secondary?” So, you are doing all those tests to look for infection, to look for an underlying malignancy and you are trying to answer the question about primary HLH by doing your natural killer assays and also, by doing genetics. And deal the initiating cause - treat the cause - particularly if you have EBV, give rituximab.

What about if they need salvage? So, what about relapsed refractory HLH. The data for this supports ruxolitinib. Ruxolitinib is a JAK inhibitor. Now, remember we had our little signal men, the cytokines going, "Beep beep, beep beep, beep beep, beep beep, beep" sending out the messages. Well, the message pathway they use is a JAK pathway. And so, you can jam the message pathway by giving ruxolitinib. And actually, this is a really exciting advance as well. It was first demonstrated in a 11-year-old boy, and there are now over 18 papers. And when you try and work out which patients are duplicated in these papers, there are about 115 patients. And there is ~ 70% response rate, which is a really good response. So, this is useful. It also seems to be quite good with cytopenia. And in about 24 to 48 hours, you are seeing an increase in the counts, which is interesting because those of us who use ruxolitinib in the context of GVHD, we worry about counts, especially platelet count. But actually, in HLH you see the counts coming up, you see the fever settling. The main problem is it is not always sustained as a response. In somebody like this, you have to make sure that you are going to give it for enough time with enough of the adjunct therapies to see that response continued.

The other treatment, just to mention here, is Emapalumab, which is anti-interferon gamma. It is licenced in America. It was licenced in 2018 by the FDA in primary HLH. And there are now studies coming out using it in secondary. So, in primary it is brilliant. So, let us just put it little arrow around there to having it as a possible relapse therapy up. But it has not yet been demonstrated to be definitely good in secondary HLH.

So, that is my little schema for using the therapies in HLH. And that brings us very nicely to the end of the talk.

Dr Phillips: Thank you very much. That was absolutely magnificent. I am flicking around to try and make this Q&A box open for me. And while people are gathering themselves from the onslaught of genius that they have heard, I wondered if I could ask a few questions and demonstrate my sheer ignorance of loads of stuff. To go back to the differentiation syndrome stuff with the wiggly things going through and zooming along really, really, really quickly. In the setting of APL, the description sounds like septic shock. I get a call about a patient like this, they might have APL but they might have raging Klebsiella sepsis, E. coli sepsis,

something like that. So that then makes me really worried to start slamming in large doses of Dex in this setting. Can you give Dex and Piptaz or meropenem at the same time?

Dr James: Absolutely. I think if you think they have an infection you need to be cracking on and giving your broad spectrum antibiotics, but don't withhold the Dex.

Dr Phillips: Yep.

Dr James: And you will see them turning around and so now you have the question, ”well, which was it that made the difference? Was it the steroids? Was it the infection?” But actually, you will also be getting back your results, your blood results back which will also be helping you differentiate between the two.

Dr Phillips: Yeah, yeah.

Dr James: As we are on differentiation syndrome, one thing I didn't mention was the fact that we now give prophylaxis. The jury is slightly out as to whether it is or isn't a good thing. In paediatric practice, in the new UK guideline, we have opted to give prednisolone prophylaxis 0.5 mg/kg from days one to 14, for the first two weeks in both low risk and high-risk patients...

Dr Phillips: And that's steroid? A drizzle of prednisone.

Dr James: Just a little drizzle of steroid as we go along from the start. You give the ATRA first absolutely. And remember, this hand is calling for ATRA, this hand is calling for blood products, *and* get going with the prednisone. The PETHEMA group give dexamethasone, they did look at giving prednisone, but they give 2.5mg/m^2 dexamethasone bd - half the dose that you give in treatment, again, for the first 15 days. We know there are some groups who are particularly high-risk, so some give it to the high-risk groups. If the white cell count is >5 at presentation, you are more likely to have DS. If your white cell count is increasing rapidly as you're giving the ATRA and the arsenic, you are likely to have it. There is a little peak at one week after starting, another little peak three weeks after starting. But by the time you have got the counts coming down again, you are through the worst of it. If you have renal injury, you are more likely to have differential syndrome. And interestingly, if you have a high body mass index, you are more likely to have differentiation syndrome.

Dr Phillips: I mean, it makes sense with the rising counts, it makes sense with the higher numbers of white cells because they're going to differentiate through faster. But why if you’re just bigger?

Dr James: Well, we don't know, but it's probably or possibly because of two reasons. One is because you are going to have far more problems if you start to gain fluid because over a certain level, having a higher body mass index is a kind of comorbidity. And so, if you start to become oedematous and put on weight, you are more rapidly going to struggle. But, secondly, it may also be because we have not got the doses right because we have not done enough PK studies in people who have a high BMI to know whether we are completely overdosing and so, causing more problems. We don't know the answer.

Dr Phillips: Yeah, with it being described and you've got the cytokine stuck around the outside and you've got IL-1, IL-6 and the sort of pro-inflammatory stuff going on and knowing that we've used the sort of the anti-cytokine drugs within other sort of over inflammatory settings. Is there work, is there an option to go in with tocilizumab and friends like that within differentiation syndrome?

Dr James: Yeah. Well, it is really interesting. You might think, "Well, you have got TNF alpha, why aren't you giving etanercept? Why aren't you using those things?" I suspect it is because it is relatively rare and so, there just has not been a call for it. But you look at it and you think actually these are really all very similar. I actually brought long two play figures that a colleague lent me. I was thinking this Hulk figure is a bit like what happens in the hyper-inflammatory phase where there is this sort of overactive, "I've been set off now and I can't switch myself off so I'm just kind of rampaging around" problem. But then, I thought maybe

it is actually a bit more like this Spiderman because the white cells are actually sticky. It's a very sticky problem you have, so, I think it is a bit more like Spidey. But the actual pathway becomes very common for all of these things. And it would make a lot of sense to me to actually use those, but it usually turns off pretty quickly with steroids.

Dr Phillips: Yeah, yeah. So, almost, if Dex in large doses is working really quickly then sort of it's a bit overkill or extra faff to try and find something else that may not really make a difference in the first place. And the other thing I was thinking with you talking about like the rising up and the numbers and stuff like that. When we've got someone with a massively bulky Burkitt or a really, really high-count infant leukaemia, we go really gentle to start with and then slowly build up. Is there actually a call for using that approach with the ATRA and arsenic rather than going smack old bugger, smack with steroids?

Dr James: Yes, it may well be that we find out we can get away with a smaller dose of ATRA at the start and just try and control the whole process more. But then, again, we are getting it right for most people, so, if you have a kind of normal distribution, actually if you are getting it right, it is going to have a bit at the end with people who are still getting problems. So, it is about recognising who is at risk of being at the end and recognising when people are slipping into DS, and then kind of going, "Okay, let's just control this as we go out a bit more."

Dr Phillips: So, it's sort of that argument a bit like the antibiotic prophylaxis in leukaemias and everything else. It is how much do we affect a little bit here versus being reactive and causing less problems for the less people across the whole of this population.

Dr James: Yes, because the price we pay for treating most people really well is that a very small minority will get DS.

Dr Phillips: Yeah. That is really, really fascinating and has helped me with that loads. And I would encourage people to as you can see the questions that are coming through are not really deeply intellectual, so feel free...

Dr James: I can't see any questions at all actually.

Dr Phillips: No, I haven't seen any coming through yet and it could be that I've gotten this wrong. Which is great because that gives me opportunity to ask even more.

Dr James: Maybe I was just on mute for the whole thing.

Dr Phillips: No. But if people do have questions, please do. I was also thinking as you were talking about the HLH side of things. Loads of different sort of questions around. And I wondered a bit on the diagnostics actually. When you talk about going back into the drugs and history and the stem cell transplantation risk factors, how far back do we go? Is it drugs that they're taking at the time or is it a fortnight ago or is it in the last year? And same with transplant, is it before they've recovered their white cells? And what's the sort of worrying times for secondary HLH kicking off?

Dr James: So, I guess there are different answers. With transplant the issue is probably that you have got a kind of acquired immune deficiency. And so, that is most likely to be a problem in the early period and that is also interacting with the fact that in that period you are most likely to get infection, which may also trigger it.

Dr Phillips: Yeah.

Dr James: So, most of the transplant cases occur in around the first 90 days. And it is more likely to occur if you have got GVHD, or if you have got TMA. It is that kind of downward spiral that you see in transplant

patients I am either doing really well, or now actually getting problems -and then one problem leads to another problem. With the other group, was it the drug triggered HLH?

Dr Phillips: The Drugs. Yeah.

Dr James: So, the drugs, well I have not seen this very often although HLH is an interest of ours and we see quite a bit of genetic HLH. We did have a case that was devastating and it was probably triggered by a drug that had been given, but had stopped by the time they came into hospital with the HLH. So, it took a while for us to work out that that was what had triggered it. But because once you set HLH running, even if the precipitating cause is gone, you cannot necessarily turn the whole thing off. You can still get full blown HLH. So, I would think you would be looking at something fairly recent that is likely to have been given within the preceding eight weeks.

Dr Phillips: Yeah, and so we're not just saying what did you come in taking, but we're actually thinking...

Dr James: Have you had anything new?

Dr Phillips: You're sick. What did you do recently? Even if it was for a short course or something, or you started it and you switched onto something else. That might be the thing that's toppled you over. Which I guess then makes me wonder about that idea of chasing the original cause to typically roll over into that spiral. Is the idea behind trying to fix it, be it a malignancy or something else, to stop it carrying on pushing it?

Dr James: Yeah, it is because as long as that is untreated, you are likely to be constantly aggravating the course. It is also because that is often a really important thing to have treated in and of its own right. So, certainly with the EBV associated ones, you want to be turning that off in virus associated HLH. With the LPD associated HLH, actually, it is usually a little way into treatment and it is more to do not directly with the leukaemia or the lymphoma, but it is to do with the acquired immune deficiency they have got. So, you have usually got time to pause, treat the HLH, then, go back to treating the underlying disease. Quite frankly, you are unable practically to treat the leukaemia lymphoma anyway when they middle of raging HLH. With the autoimmune HLH, again, the things that are treating the HLH, the methylprednisolone and the anakinra are going to treat the underlying autoimmune cause. It is partly about you trying to answer the question, “what has caused this? How can I work it out?” Because one big question to try answer is: “is this person somebody who is going to need to get to transplant”. That is particularly the issue in the children. You are always trying to think “is this a primary?” because we know that probably the thing that affects your survival is getting to transplant. And so, the sooner you get to transplant, the better we think you will do, because you are less likely to relapse and have a problem before you get to transplant. There are certainly patients who historically never got to transplant because they relapsed as there was no urgency, it was not seen as an emergency. But actually, HLH, primary HLH *is* an emergency in terms of getting to a transplant. So, that is the other reason you try and answer that question. Now, just because you find an infection doesn't mean you have not got a primary HLH, but it does mean you understand what triggered it in that patient.

Dr Phillips: Yeah, yeah. Your primary HLH could have been sat there waiting just about managing to hold itself together and then comes along your EBV, CMV. Yeah.

Dr James: Exactly. Because they're like time bombs really. Just waiting for something to unmask the fact that they can't kill the cells they need to.

Dr Phillips: Yeah, yeah. And then it's toppled over the edge, yeah. And I guess as we learn more, we'll be able to work out things like why it seems to be the herpes viruses, why it seems to be certain things that are more likely to trigger than others. Because of the pathways involved, I would guess.

Dr James: And one of the things we don't understand yet is to what effect the maternal antibodies that cross the placenta give some protection against it because it is not necessarily (sometimes it is) the first infection the child has got that then causes the primary HLH. Sometimes, it happens later on. What is it that protected them, what are the epigenetic factors that protected them? Why did they present so late in those who present when they're a bit older?

Dr Phillips: Yeah, and as you were saying with this sort of group that present with ?sepsis, that might be the trigger. But they're another group, aren't they? Where it feels like you can't help but treat them for raging infection whilst you're also considering removing what little of their immune system is left working with Campath, massive doses of methyl pred and cyclo or variations on that theme.

Dr James: Yes, and I think the other thing to stress is that usually actually with both the HLH patients and with the differentiation syndrome, we usually see a very rapid response. So, that you can have somebody who is almost moribund, for whom a few days later you begin to think about plans for home. They can turn around very quickly. And if they are not, then obviously go back, think about your diagnosis, think what you have missed, look at your treatment. If you're sure of your diagnosis, move on to your next line treatment in many cases because these are such aggressive situations that you have not got time to just take a long view on the whole thing.

Dr Phillips: Yeah, so it is that sort of, it's proper daily review with consideration of changing the treatment plan at 24, 48 hours and so rather than giving them time and space to keep working away. I guess my only other question that I was wondering on this, was that... With all of these differentials going on, is there any value in sort of the differential ability of other blood tests to separate things? Like when we see severe sepsis, we're using lactate to try and drive us one way or procalcitonin or the inflammatory markers. Do they have any value in helping us differentiate these things?

Dr James: Yeah, so I think of the things that really help you lactate is very good actually, because often with HLH, the lactate is ridiculously high. So, you have a high lactate, I've seen it almost up to 30. And even in severe sepsis, there is usually an improvement in lactate when you give fluids,when you get the blood pressure up, when you give antibiotics. But you do not see it improving in HLH, so, that can be quite helpful. The genetic screens and the natural killer assays can be very, very helpful. So, you perform the assays, SAP, XIAP. And if you are in a good centre that does them, that is very helpful. If not, then make sure you have got links to a centre that does and you know the pathway - that you know when to send them, that you phone the lab. All those little things that make it robust. Make sure that you know how to send them, that they are going directly there, that they are expecting them, check they have arrived, that you know when you are likely to have result, because they can make a big difference.

The cytokine pathway: I didn't mention this in the HLH situation, but the cytokines are actually very similar. You have got IL-1, IL-6, IL-18, TNF alpha, and interferon gamma, hence the emapalumab. In some places people have access to doing cytokine profiles very rapidly. They might also suggest to you that you have got an HLH patient on your hands rather than it just being infection. But I don't think we really know enough about the changes between those. Probably, the platelets and the ferritin are very helpful because of the rapidity. There are very few things that cause a ferritin to increase very rapidly, so that is very discriminatory from the start. You might have a ferritin that goes from just... 600, next day it's 2000, next day it's 30,000. That is not infection. HLH is the condition *par excellence* that causes an incredibly rapid rise in ferritin. So, even if your laboratory is not happy, just phone them and say, "Look, this will make a real difference clinically." Let them know if your patient is jaundiced and that there will be bilirubin interference as they need to do it in a special way. And you do not just want one that says >1600 or whatever your lab does because the ferritin is very discriminatory. Having a platelet count that is falling is also discriminatory because it is unusual in an infection for the platelet count to fall, it usually goes up. It can fall in severe

sepsis, but usually it goes up rather than down, so if that is falling, they may have acute sepsis or it may well be the HLH. When it comes up, it is also a great marker.

Someone in the chat has written about infection. Leishmania is a, very, very good mimic of HLH and then you can also get Leishmania-induced HLH just to confuse the picture. So, you address both hand in hand actually. It is a very good question. You approach it hand in hand. You absolutely treat the infection, and you treat the HLH the same time. You know it is a spectrum. If you went to intensive care, in paediatrics certainly, and you did HLH screening on all the children there, you would probably find a lot of children who met or almost met the criteria because they are on that pathway from having a healthy inflammatory response to being completely out of control. And a lot of them turn around by themselves once you begin to treat the infection. Some of them do not need the full-blown treatment, they might just need some steroids, and then they start to come down. So, some of it is about just judging what is going on. But you need to make sure you have got very good communication pathways, and that if there is any suggestion that the HLH is going out of control, because you are not treating it well enough, that you absolutely give full treatment. Leishmania obviously, you may well see down the microscope as well if you are looking at bone marrow and you see the Donovan bodies. And so, one of the questions that we always ask ourselves, looking under the microscope is, am I missing Leishmania? Because that is a kind of a classic mimic.

Dr Phillips: Thank you. We've got a couple of minutes left for anybody else to throw extra questions in, but I've certainly found this very, very helpful and…

Dr James: I actually just found on my desk, one of my lovely parents knitted me a neutrophil, so I just thought I would share that with you as well.

Dr Phillips: Oh, that's lovely.

Dr James: The beauty of working with paediatrics?

Dr Phillips: And now I think I quite like the idea of what we're really dealing with is a Hulk that is out of control. A proper, red-eyed Hulk or bad Spidey perhaps going around and doing naughty things.

Dr James: And you can just imagine if you have clumps of white cells sticking together, sending off beep, beep beep, beep, beep, beep messages out of control, releasing something that's going to also just damage the endothelial. That's like sort of sniper fire with the Cathepsin G. And then you've got these big, and then, the endothelium part because it's no longer able to be intact together. You're marauding white cells go through and these big clumps get into your tissue. As they have made that big gap in the endothelium, you get fluid coming in behind them. You can see why someone gets incredibly sick. Transplant as soon as possible. So, if we have somebody where it is very likely to be a primary, we would hope to have made that diagnosis by the end of the first four weeks. We take tissue typing as we are making the diagnosis of HLH. So, think about HLH, if you think there is a possibility it might be primary, do tissue typing, get the siblings up, test the parents and then we would aim to have made the donor choice by about week seven of treatment, so certainly by the end of induction. And we will be aiming at about week eight or so to be starting with conditioning. And that is because we have seen children relapse when they were on the way to being worked up for transplant. Even when we were being fairly prompt about it, we have seen children relapse before they get to transplant. And you just want to avoid that. We have also now seen children be transplanted very early and do very well and escape lot of the other problems that they might have had. And actually, we are running a study from here looking at the sort of optimum interval between diagnosis and transplant.

Dr Phillips: Sort of wait them to get them better enough to get transplanted but not long enough that it can flare up again in the cases of primary.

Dr James: Because they are time bombs because you have not changed underlying problems. So, as soon as they meet another infection, the whole cycle will start. And actually, it often seems to be more aggressive each time.

Dr Phillips: Yeah, yeah. It's like it's learned but it's learned badly to do the wrong thing. Well, it's now quarter past six in the UK, quarter past seven, I think, in central Europe. And thank you for a really extensive and detailed, and paediatrician friendly, because I understood that, a conversation on two complex but interrelated topics. I hope that people do come back and watch this again as they're going forwards in their careers, and we get people who have been better treated for their HLHs and their differentiation syndromes because of that. Thank you very much, Dr James.

Dr James: Thank you.

Dr Phillips: And thank you very much to the organisers.

Dr James: Thank you, Bob.