The only thing that we can know is that we know nothing, and this is the highest degree of human wisdom. Pierre Bezhukov, ***War and Peace***

This month we turn our eye for the humanities away from music and towards literature. Our focus on literature will be Leo Tolstoy's sprawling masterpiece, ***War and Peace***. The quote above is the culmination of Pierre's musings on the meaning of life: where he reflects on a litany of questions, only to find that his reservoir is empty when it comes to their answers.

The only thing that we can know is that we know nothing. This statement can certainly be construed as a defeatist sentiment but if one traces its roots further in western philosophy, we end up with Socrates and his view that wisdom begins from the wonderings that arise after one admits ignorance on a subject.

How then do I transition from a discourse on nothing to the world of Diamond Blackfan anemia? Well, in a way, as my clinical colleagues tell me, a diagnosis of DBA is typically based on nothing: or perhaps to phrase this statement with a little more sensitivity, DBA is often a diagnosis of exclusion. Basically, what this latter statement means is that after other possible diagnoses are excluded, one is left with DBA.

While this view of how DBA diagnoses are made is clearly an oversimplification, it does point to the fact that historically DBA has been a disease that is difficult to diagnose. This difficulty arises from the lack of a definitive test that distinguishes DBA from other possible diagnoses for a patient presenting with an aplastic anemia. For example, Fanconi anemia is an inherited bone marrow failure syndrome that is caused by a defect in a pathway by which cells repair DNA damage. Cells lacking this DNA repair pathway are unusually sensitive to DNA damaging agents, which has been exploited to create a definite diagnostic test for Fanconi anemia. Such a test is lacking for DBA.

With the identification of a host of DBA genes in recent years, genetic testing has made it increasingly possible for a physician to make a DBA diagnosis with confidence. However, genetic testing only goes so far since not all DBA genes are known and not all mutations in a gene are pathogenic. Thus, even with genetic testing there can remain a certain ambiguity in making a definitive diagnosis of DBA.

This brings us to this month's Journal Club article. A manuscript by Farrar *et al* entitled "Exploiting pre-rRNA processing in Diamond Blackfan anemia gene discovery and diagnosis" published in the *American Journal of Hematology*[1]. Conflict of interest note: I am a senior author on this manuscript, so I should be considered unduly biased in the viewpoints that follow. Be that as it may, let me continue by saying that the manuscript by Farrar and colleagues has at least one element in common with Tolstoy's masterpiece: it is lengthy and covers a considerable amount of ground. While many published manuscripts have a single major point to make, this manuscript has almost as many points to be made as authors, of which there are many.

The manuscript begins with the case of a child presenting with a bone marrow failure that was difficult to diagnose. Studies led by Ross Fisher ruled out Fanconi anemia and Shwachman Diamond syndrome based on a combination of laboratory tests and genetic testing, thus leaving DBA as a possible diagnosis based on these exclusions. Genetic testing for DBA was ambiguous with mutations in 2 known DBA genes that were considered innocuous. The patient did however have a large chromosomal deletion that contained a gene encoding the large subunit ribosomal protein, Rpl31. This was not a known DBA gene, so while suggestive, it was unclear whether the loss of a copy of *RPL31* represented the pathogenic mutation in this patient. The patient was referred to Drs. Vlachos and Lipton at the North American DBA Registry (DBAR) and a multidisciplinary effort was undertaken to determine if *RPL31* represented a new DBA gene, which in turn could contribute to making a more definitive diagnosis for this patient.

Jason Farrar and I took the lead in using pre-rRNA processing to reveal that cells from the patient had evidence for a defect in ribosome synthesis consistent with that expected for haploinsufficiency for a large subunit ribosomal protein. In this way the patient was similar to other DBA patients having mutations in large subunit ribosomal proteins. This result strongly suggested that *RPL31* was a new DBA gene. David Bodine's group took the analysis one step further by showing that reducing the expression of *RPL31* in $CD34^+$ hematopoietic stem cells prevented these cells from differentiating efficiency along the erythroid lineage; thereby explaining how a patient with a mutation in this gene could present with anemia. This result further established *RPL31* as a DBA gene and established a new standard for identifying DBA genes.

The procedure used for the pre-rRNA processing studies outlined in this work is not for everyone. In fact there are relatively few labs in the world that still do it on a routine basis. It is not that it is unduly difficult, it is just that it is messy, uses hazardous chemicals, and to top things off, employs radioactivity as a means of detection. Therefore, it was of interest to note that the defect in ribosome synthesis in cells from the *RPL31* patient was evident in the early stages of this procedure, prior to the need for radiological detection. This observation was quite a surprise as it had not been reported before and the authors began looking for whether the same was true in cells from other DBA patients containing mutations in other large subunit ribosomal protein genes. To expand the number of patients examined, we included patients from both the North American and Italian DBA Registries, the latter effort spearheaded by Irma Dianzani and Ugo Ramenghi.

The results from this expanded analysis showed that this defect in ribosome synthesis was evident in virtually every patient with mutations in known large subunit genes (the exception, a rare RPL15 sample) without the need for radiological detection. To see this defect, all that was necessary was to isolate RNA from patient cells and run this RNA out on an agarose gel. Since most laboratories have the ability to run agarose gels, this suggested a rather straightforward approach to identifying ribosome synthesis defects (at least for large subunit genes), which could in principle be exploited for use in DBA diagnosis. This approach suffers from one drawback peculiar to RNA, which is that when separating RNA on an agarose gel, formaldehyde is included in the gel to denature RNA and facilitate its separation. Formaldehyde is pretty nasty stuff with both acute health effects and chronic health effects, the latter of which includes being a suspected carcinogen. As mentioned, few laboratories use this procedure routinely, which limits its use as a diagnostic procedure.

Paola Quarello then took the lead in applying an alternative technology to fractionate RNA employing a fancy piece of equipment known as a Bioanalyzer. RNA can be analyzed with a Bioanalyzer without the need for either formaldehyde or radioactivity. More importantly, most routine diagnostic laboratories have Bioanalyzers on hand for routinely assessing the quality of RNA isolated from biological samples for forensic and other purposes. Together, the authors were able to show that the Bioanalyzer could be used to identify ribosome synthesis defects in patients with mutations in known genes encoding all large subunit proteins with the exception of RPL15 (didn't someone once say that the exception makes the rule). Importantly, haploinsufficiency for *RPL5* and *RPL11*, the two most frequently mutated large subunit genes in DBA patients, could be analyzed in this manner.

Among the major points made in this manuscript was the identification of *RPL31* as a new DBA gene. Further, as this analysis was expanded to include the Italian DBA Registry, it was noted that a patient described in a manuscript back in 1999[2] had bone malformations similar to the *RPL31* patient reported here. Subsequent sequencing of DNA for the patient described in 1999 revealed a presumptive pathogenic mutation in *RPL31* establishing a genotype/phenotype relationship between mutations in *RPL31* and this bone malformation.

While the demonstration that *RPL31* is a new DBA gene is important, I feel the broader implications of this manuscript reside in the identification of a technology routinely found in diagnostic laboratories that can be exploited for a rapid and sensitive test for ribosome synthesis defects in a fraction of DBA patients. At present, the analysis is primarily limited to patients with pathogenic mutations in genes encoding large subunit ribosomal proteins but the authors suggest that with further study this technology may also be adaptable to patients with mutations in genes like *RPS19* encoding proteins of the small ribosomal subunit.

This technology (hopefully coming soon to a diagnostic laboratory near you) promises to have an impact on DBA diagnosis similar to the impact sensitivity to DNA damaging agents has had in in the Fanconi Anemia field.

1. Farrar, J.E., Quarello, P., Fisher, R., O'Brien, K.A., Aspesi, A., Parrella, S., Henson, A.L., Seidel, N.E., Atsidaftos, E., Prakash, S., et al. (2014). Exploiting pre-rRNA processing in Diamond Blackfan anemia gene discovery and diagnosis. American journal of hematology.
2. Ramenghi, U., Garelli, E., Valtolina, S., Campagnoli, M.F., Timeus, F., Crescenzio, N., Mair, M., Varotto, S., D'Avanzo, M., Nobili, B., et al. (1999). Diamond-Blackfan anaemia in the Italian population. British journal of haematology 104, 841-848.