CHAPTER 5

DISCUSSIONS

5.1 Prevalence of POCD

Postoperative cognitive dysfunction (POCD) was reported globally in all kind of surgery. In major noncardiac surgery, authors summarize the prevalence of POCD ranging from 25-40% in early postoperative period (Green & Schaffer, 2019). Current study involving total knee arthroplasty patients reported 36.8% of severe POCD among elderly patients (Ren et al., 2022). In another study, the prevalence dropped to 15.9% at 3 months after surgery (Shoair et al., 2015). In Malaysia, there has been no reported prevalence of POCD in cardiac surgery up till now. There are fewer local researchers that are interested in this issue. Even in neighbouring countries, published reports on POCD were scarce. In 2018, Soenarto et al. reported that the prevalence of POCD following CABG was 40.7% at discharge (Soenarto et al., 2018). There are no other reports for late POCD as the study stops at patients' discharge. That early POCD incidence matches an unpublished report in our country. In 2012, a cardiac center in Malaysia mentioned that POCD prevalence in CABG patients was around 40% at discharge (Kadiman 2023). The percentage was high, hence clinicians are taking more attention to it as it may become a serious unwanted postoperative complication.

Over the years, a current study takes place to assess and report current POCD situation in Malaysia. The results revealed that POCD prevalence at discharge was 23.9%. The differences in percentage happen probably due to several reasons. Firstly, open-heart surgery is assisted with advanced machines and tools. This helps in shortening the bypass time, monitoring cerebral perfusion, and maintaining a steady flow of blood to organs. These are important risk factors that determine POCD. Not to forget the surgeons and clinicians that are getting more skilful over the years, hence can prevent unintended bleeding during surgery. Maintaining blood flow is crucial as neuroinflammation can occur that leads to a hypoxic brain and POCD eventually (Li et al., 2022).

Secondly, the usage of anaesthesia differs. Since 2012, IJN has pioneered Malaysia to use dexmedetomidine as anaesthesia during CPB. A meta-analysis has reported the benefits of dexmedetomidine in ameliorating POCD incidences (Yu et al., 2022). Previously, none of our cardiac centers used dexmedetomidine as an adjunct in anaesthesia during CPB. Considering the high incidence of POCD, dexmedetomidine has been used as standard of care for the past 10 years in this specific cardiac center. Since that, no study has been conducted to see the effectiveness of dexmedetomidine in improving POCD. Therefore, this current study proves that its usage has improved POCD by reducing the prevalence to almost 50%.

5.2 POCD and its Contributing Factors

Multiple factors are associated with POCD, from patients to surgery and postoperative complications; all aspects have their degree as contributing factors. This study proved that patient-related factors such as age and education level are associated to POCD. The mean age for POCD patients in this study is 65 years old that significantly differ from non-POCD patients with mean age of 55 years old. The older age group has been proven to link with early and late POCD (Yang et al., 2022a). Given that older age was also predisposed to dementia, we need to distinguish between POCD and the preclinical stage of dementia (Yang et al., 2022a). Those with mild cognitive impairment prior to the surgical procedure may worsen after surgery. Hence, screening patients to filter out those with MCI is of the utmost importance. This is to ensure that any cognitive decline that happens after surgery is due to POCD rather than worsening MCI to dementia.

Education level plays a role in assessing POCD. Since the assessment of POCD involves many mental tasks, an individual's mental capacity may affect the test scores. The term 'cognitive reserve' indicates a person's mental capacity. Exposure to a difficult task or challenging mental situation improves neural capacity, efficiency, and ability to compensate by recruiting additional regions in the brain (Tucker & Stern, 2011; Gaspar et al., 2022). Eventually, it strengthens the brain and becomes more resistant to cognitive decline. Hence, after experiencing surgery, the brain damage that occurs has a different effect on a person's cognitive state depending on their cognitive reserve. However, there are no standards for determining a person's cognitive reserve. Researchers concluded that formal education and IQ level may become the standard proxies for cognitive reserve,

although it varies across the literature (Malek-Ahmadi et al., 2017). But now, many questionnaires have been developed and validated to assess cognitive reserves (Nogueira et al., 2022). Apart from that, other activities provide evidence of improving cognitive reserves, such as work skills (Vance et al., 2016), leisure activities engagement (Iizuka et al., 2019), and social interaction (Fu et al., 2018). Most studies use education level as an objective reference to cognitive reserve, however, we should not neglect the fact that people with no formal education may have other brain-engaging activities. Their mental challenges in everyday activities may help them maintain their brain from cognitive decline. In this study, the median of our POCD patients falls in the secondary level of education.

Besides, comorbidities with other diseases showed evidence to associate with POCD. Diabetes, hypertension, chronic kidney disease and obesity was seen to correlate with POCD. However, a meta-analysis found that hypertension has no association with the risk of POCD (Feinkohl et al., 2017a). Of the 24 original articles they studied, 21 have poor definitions and assessments of hypertension, as well as varied POCD assessments. When conducting subgroup analysis, they found out that the risk of POCD increased by 27% when the study had a proportion of males >75%. Hence, they suggest that risk analysis in a subset of patients is important to confirm hypertension as contributing factor (Feinkohl et al., 2017a). Referring to our prevalence of cardiac surgery, a similar trend was found, with male patients being higher than females. We come to believe that hypertension does become a contributing factor of POCD in line with a high proportion of male patients. Our results support the hypothesis where all our POCD patients were hypertensive. Apart from that, half of hypertension patients are also affected with insulin resistance, which is proven

to impair cognitive function. In those patients, their cognitive function might affect in two ways, directly through cerebral blood flow alteration and indirectly by the associated inflammatory response (Craft, 2009). Another meta-analysis proved that the risk of POCD is getting higher in diabetic patients, especially in those who have poor glycaemic control (Feinkohl et al., 2017b). Our study found that 42% of our diabetic patients developed POCD. Hence, this study concurs that patients with hypertension and diabetes possess a significantly higher risk of developing POCD.

Other than that, the type of cardiac surgery was also an important contributor. It is well-known that cardiac surgery poses a higher risk than non-cardiac surgery for POCD (Evered et al., 2016). However, different types of procedures affect cognitive function differently. The current study compares single CABG procedures, the combined procedure of CABG and valve surgery, and valve surgery without CABG. A significant difference was found between these procedures, where patients undergoing combined procedures were the most to develop POCD. A combined procedure may possess a higher risk than a single procedure. We hypothesize this for a few reasons. Firstly, the time taken for the surgery is relatively longer. This will lead to increased bypass and cross-clamp time and a longer duration for anaesthesia. Depth of anaesthesia was known to affect brain function. Secondly, blood flow is disturbed postoperatively by two means: the bypassed artery graft and new valves. Replacement of new valves triggered silent cerebral microemboli events in 80% of patients (Pagnesi et al., 2016), which were feared to worsen cognitive function. Microemboli are the traditional way to induce mechanisms of brain injury, which correlate to cognitive outcomes (Patel et al., 2015). We suggest that there is a chance of disturbance

in blood flow to the vital organs at this point. What worsens the condition is the amount of blood loss during surgery as well as the patient's ejection fraction. The amount of blood loss in POCD patients is significantly higher than in non-POCD patients.

5.3 Differently Expressed Genes in POCD

Patients who experienced cognitive decline have a different gene expression. The DEGs may explain how POCD occur. Studies previously explored microRNAs to see how they relate to POCD. Yazit et al. (2020) summarized the microRNAs that related to POCD which includes miR-572, miRNA-155, and miR-181b-5p. There were also evidences of non-coding genes like long non-coding RNA (lncRNA) and circular RNA (circRNA) being associated with POCD (Zhang et al., 2018; Wang et al., 2019). Despite the evidences in non-coding genes, we intended to elucidate coding genes that related to proteins that potentially use as biomarkers.

5.3.1 KIR Genes

Three of five significantly expressed genes found in POCD patients from Comparison 1 were immunoregulatory. Killer cell immunoglobulin-like receptors (KIR) are protein-coding genes. Two characteristics classify them – 1) extracellular immunoglobulin number, either 2D or 3D, and 2) length of the cytoplasmic domain, either long (L) or short (S). The current study identifies three KIR genes which are KIR2DS2, KIR3DL2, and KIR2DS3. Briefly, these genes are receptors that are attached to natural killer (NK) cells and human leukocyte antigen (HLA), their ligands. The specific function of NK cells differs depending on the receptors attached. Basically, NK cells function to kill cancerous cells or cells

infected with the virus. KIR, on the other hand, is a regulatory receptor that activates or inhibits the activity of NK cells (Pende et al., 2019). The current study revealed that all three downregulated KIR were inhibitory KIR genes.

There was a study on NK cells with KIR2DS2 immunogenotype and patient-derived glioblastoma (GBM) and gliosarcoma cells. They discovered that KIR2DS2+ NK cells demonstrated higher cytotoxicity towards all GBMs (Navarro et al., 2014). The KIR2DS2 genes could identify alloreactive NK cell subsets with greater potency against GBM. Hence, they suggested that intrinsic cytotoxic potency is the mechanism of NK cells with KIR2DS2 immunogenotype towards GBMs (Navarro et al., 2014). Besides, there is evidence of KIR2DS2/KIR2DL2/HLA-C1 haplotype combination that correlates with lower MMSE scores, representing the condition of Alzheimer's disease (Rizzo et al., 2019). For KIR2DS3, a study founds a significant correlation between KIR2DS3 and acute myeloid leukaemia (AML). The frequency of KIR2DS3 was significantly lower in the AML group compared to the control (Shahsavar et al., 2010). We speculated that low expression of the genes reduces the potency of NK cells against AML cells. Other than that, KIR3DL2 was found to be an independent prognostic factor and biomarker for Sezary syndrome (H Hurabielle et al., 2017). The KIR3DL2+ cells percentage of more than 85% at diagnosis is associated with reduced disease-specific survival. In amyotrophic lateral sclerosis (ALS) patients, astrocytes with KIR3DL2 receptors correlates with motor neuron death. The knockdown of the receptors has resulted in enhanced motor neuron death (Song et al., 2016). To conclude, KIR genes may enhance or restrict NK cell activities and affect the targeted cells.

5.3.2 How KIR affect POCD

The pathway generated from these gene lists proves the involvement of the KIR genes in antigen processing and presentation pathways via the regulation of NK cell activity. Specifically, KIR3DL2, the inhibitory receptors in humans, were found to be involved in natural killer cell-mediated cytotoxicity. The endpoint of this pathway is cell apoptosis. Hence, any pathway changes may promote or inhibit target cell apoptosis. In the case of POCD patients, the expression of KIR genes was downregulated. We come to believe that this downregulation of inhibitory genes will promote the activity of NK cells. As a result, NK cells mediated cytotoxicity will cause damage to neighbouring brain cells. The specific mechanism is yet to be confirmed. However, with more brain damage occurring, the likelihood of cognitive changes in patients after surgery increases.

In another study, researchers revealed that NK cells have a role in Parkinson's disease (PD). NK cells were found to inhibit microglial transactivation, which eventually reduces neuroinflammation (Waggoner & Kumar, 2012). In PD pathogenesis, it is speculated that NK cells possess a neuroprotective effect (Navarro et al., 2014). Neuroinflammation is a well-known mechanism that leads to POCD pathogenesis. Hence, the authors hypothesized that the downregulation of KIR genes may relate to neuroinflammation by interfering with the neuroprotective effect of the NK cells (Yazit et al., 2023). These KIR genes' specific mechanism and association towards neuroinflammation were still vague and need further research.

5.3.3 BTNL3 and Cytokines

Aside from KIR genes, we discovered that butyrophilin-like 3, BTNL3 genes were downregulated in POCD patients compared to non-POCD patients. It comes from the subfamily of butyrophilins (BTN), a transmembrane glycoprotein from the immunoglobulin superfamily. They are commonly found in milk fat globules (MFGs) and involve in lactation (Redwan et al., 2018). However, polymorphism of the genes was associated with the pathogenesis of several diseases. Specifically, BTNL3 was one of the significant genes found in monocytes and microglia in an Alzheimer's study (Patel et al., 2021). However, despite the actual function being unknown, the deletion of BTNL8-BTNL3 has been associated with TNF and ERK1/AKT pathways. The pathways are crucial in apoptosis, immune regulation inducing inflammation, and proliferation. Hence, it is suggested that the deletion of BTNL8-BTNL3 may be correlated with inflammatory diseases (Guo & Wang, 2015). The authors also suggest that BTNL3 are immune-related (Patel et al., 2021). The fact that the gene is downregulated, may experience the same effect as KIR genes due to the immunosuppression. The information and research regarding this gene in specific are very scarce, hence we could not elaborate on their possible association with POCD further.

5.3.4 Erythroferrone

Other than immunoregulatory genes, one more gene of interest was dysregulated. Erythroferrone, or ERFE, was found to be upregulated significantly in POCD patients. ERFE, previously named Fam132b, comes from C1q/tumour necrosis factor (TNF) superfamily. It functions as an erythroid regulator of hepcidin, a hormone that controls plasma iron levels and total iron content in the body (Srole & Ganz, 2021). Two conditions regulate ERFE production. First, it is stimulated by the erythropoietin (EPO) hormone in the kidneys, especially in response to low red blood cells, for example, anaemia. Another factor that might be as important as EPO stimulation is the availability of cells that secrete ERFE in the bone marrow. Hence, ERFE production is proportional to the EPO stimulation and number of erythroid precursors. In disease situations, the production of ERFE is greatly expanded in beta-thalassemia or Cooley's anaemia. In physiological conditions, ERFE production is triggered by blood loss through bleeding and hemolysis. In both cases, anaemia occurs, stimulating the production of EPO in the kidneys. ERFE production by erythroblast will then increase in the bone marrow, be secreted in blood and travel to the liver (Kautz et al., 2014). The liver will suppress hepcidin production, allowing iron from stores to mobilise to the bloodstream. In short, in response to lack of blood, ERFE production increases to suppress hepcidin, increasing total iron in the blood. Iron availability helps to increase oxygen supply in response to hypoxic conditions (Coffey & Ganz, 2018).

5.3.5 ERFE and POCD

Regarding POCD, we investigated the upregulation of ERFE associated with cognitive decline. Blood circulation to the brain in post-surgery patients may affect their cognitive functions depending on the areas of the brain. It is well-known that cerebral autoregulation is a risk factor for POCD (Yang et al., 2022a). This is due to reduced blood flow to the brain, causing the brain to be poorly perfused. Specifically, we suspect much blood loss that causes low blood pressure in POCD patients. As a result, a poorly perfused brain may experience cerebral hypoxia. The greater the blood loss, the higher the amount of erythropoietin to be released to compensate for the loss. Since EPO was one of the factors that increased ERFE production, ERFE genes will be highly expressed to produce more erythroferrone. This will suppress hepatocyte hepcidin production, increasing iron mobilisation from stores and dietary guts. Eventually, an increased amount of iron and erythroblast produced will regain the blood loss. To sum up, ERFE was highly expressed in POCD patients as they need blood. This matches the role of ERFE in systemic iron homeostasis. We illustrated how ERFE relates to POCD in Figure 5.1 below.

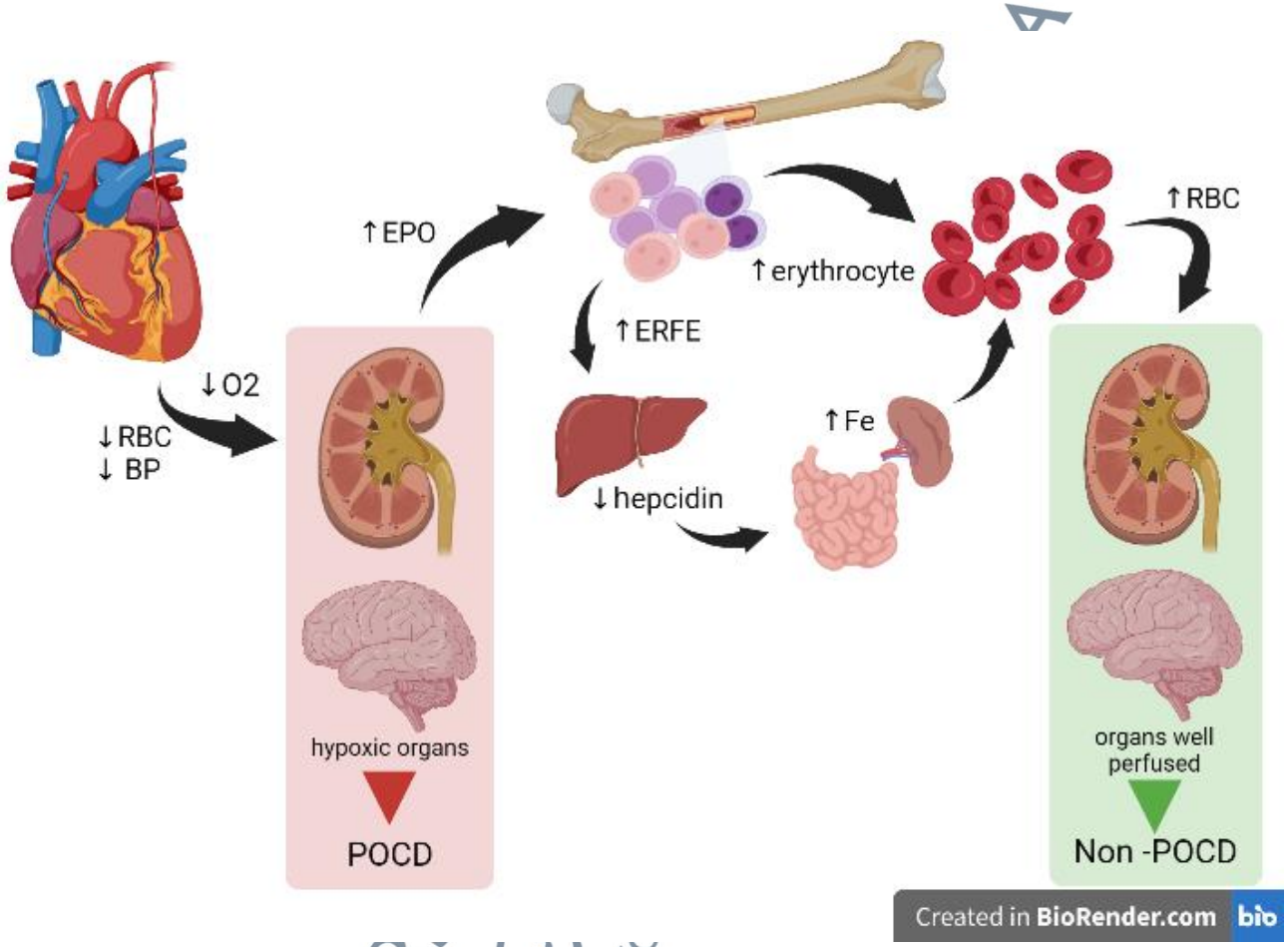

Figure 5.1: How POCD affect ERFE production in systemic iron homeostasis. Following cardiac surgery, blood loss increases, leading to reduced red blood cell, low blood pressure and low oxygen tension. This causes vital organs to be hypoxic. Hypoxic brain increases the risk for POCD. Low oxygen tension in kidney will trigger erythropoietin production. High EPO lead to increase erythroblast production in bone marrow. Erythroblast secretes erythroferrone that will act on hepatocytes to suppress hepcidin production. Hence, inhibition of hepcidin results in mobilization of iron and transferrin from cells and dietary guts into the bloodstream. High iron and erythroblast will increase red blood cell production, hence restoring blood oxygen tension and blood pressure. Risk of POCD decrease when organs are well perfused.

5.4 Cerebral Oxygenation to Prevent POCD

When discussing open heart surgery, one of the main issues to be highlighted is blood circulation. It is of the utmost importance for clinicians to maintain that blood flow to organs is well maintained throughout the bypass procedure to ensure organs are well-perfused and not hypoxic. In the current study, we investigated the genes involved in patients that utilised cerebral oxygenation monitoring.

Regarding blood circulation and its close relation with POCD, monitoring organ perfusion becomes necessary. Clinicians use regional oxygen saturation (rsCO2) monitoring to monitor cerebral perfusion as the brain was claimed to be an 'index' organ (Jo et al., 2020). The intervention is able to elucidate cerebral oxygenation, hence explaining the incidence of cognitive decline that occurs in patients with different genes expression. One of the genes we found interesting was MORN4 (MORN repeat containing 4) genes. MORN4 is a protein-coding gene that are involved in axon injury. In an animal study, MORN4 enhanced axonal degeneration in the fruit fly *Drosophila melanogaster* and mouse sensory axons (Battacharya et al., 2012). Interestingly, MORN4 also promotes axonal degeneration in mammals. These axonal injuries have been identified as early events for degenerative diseases such as Alzheimer's and traumatic brain injury (Graham et al., 2020). Hence, downregulation of MORN4 might reduce axonal injury, reducing the risk of neurodegenerative diseases and perhaps cognitive problems.

Another downregulated gene is C-C motif chemokine ligand 23 (CCL23). It is a chemokine-secreted protein involved in inflammatory processes and immunoregulatory responses. CCL23 has been widely established to play a role in either the development or

progression of inflammatory diseases (Simat et al., 2018). In diseases like rheumatoid arthritis, atherosclerosis, and systemic sclerosis, CCL23 was found to be elevated (Kim et al., 2011; Yanaba et al., 2011). It is also associated with brain damage and stroke. A study about mild cognitive impairment (MCI) and Alzheimer's disease (AD) revealed that the CCL23 plasma level is high, which suggests its relation to an increased probability of progression from MCI to AD (Faura et al., 2020). Other than that, G protein-coupled receptor 37, like 1, GPR37L1, was also significantly downregulated. The gene was exclusively expressed in the brain and associated with neuroprotection, seizures, and cardiovascular diseases (Coleman et al., 2020). However, the exact mechanism is still scarce. With all the evidence, we conclude that the downregulation of MORN4, CCL23 and GPR37L1 in our patients is beneficial. In patients that utilised oxygen monitoring during bypass, all the mentioned genes help reduce brain injury, reducing the risk for cognitive decline. We believe that implementing cerebral oxygenation will benefit the brain as a whole, not only in terms of cognitive state.

5.5 Surgery and DEGs

High-risk surgery like CABG impacts an individual as a whole, including their molecular level. The genes were expected to be differentially expressed. Our analysis revealed that there are 456 dysregulated genes. Out of that, none of the DEGs in POCD patients matched those in normal postoperative patients. The same goes for DEGs in patients utilising cerebral oximetry, except for one gene, SMPD3. Sphingomyelin phosphodiesterase 3, or SMPD3, are genes that produce enzymes, which are important in forming exosomes in multivesicular bodies (Trajkovic et al., 2008). DEGs in patient's post-surgery were mainly involved in DNA and RNA build-up, transcriptional activation, regulation of cell apoptosis, tissue morphogenesis, etc. In short, the essentials for cell regeneration and repairing mechanisms were the most expressed. Other than that, no genes are similar for all four comparisons we analysed in the current study. Hence, we conclude that genes expressed in POCD patients and patients with cerebral oximetry were exclusively due to the mentioned conditions. We believe that the genes expressed were involved in the development or pathogenesis of POCD in their own specific mechanisms. Even though the involvement might or might not influence POCD on a big scale, we have elucidated some possible ways the gene may associate with POCD. Further, this clarification may help researchers and clinicians in improving POCD in patients.

5.6 ERFE as Biomarker for POCD

Based on the gene expression analysis and clinicians' expertise and judgement, the protein ERFE showed potential as a marker to predict the incidence of POCD. A high level of ERFE reflects a higher need for erythropoiesis which may indicate significant blood loss. The condition of overproduction of ERFE has been previously reported in patients with ineffective erythropoiesis, hence, serious attention must be given to its increase (Srole & Ganz, 2021). We found that the serum level of ERFE at 3 days postoperative is increased in patients with POCD at 1 week postoperative.

The amount of blood loss intraoperative between POCD and non-POCD patients significantly differs, where POCD patients experience more blood loss than non-POCD patients. This finding from our data matched our theory that in POCD occurrence, patients undergo a greater blood loss, reducing oxygen tension in blood circulation, thus indirectly increasing ERFE expression (Pittman, 2011). We believe this finding is truly beneficial for clinicians and researchers.

Further analysis of ROC curve on ERFE protein revealed a moderate performance in predicting the occurrence of POCD. With threshold at 0.761, the specificity and sensitivity were at 80% and 50%. Utilizing ROC curve is widely used in medicine including clinicians to evaluate the accuracy of diagnostic test in the past four decades (Hajian-Tilaki, 2013). The graphical representation can be used to evaluate the test performance, particularly in the determination of biomarkers. By plotting the true positive rate and false positive rate at multiple thresholds, we can see how well the test can distinguish between two groups of POCD and non-POCD. Determination of threshold value is made possible by ROC curve. Clinicians can decide the optimal threshold that balances the test's sensitivity and

specificity. The area under the curve, AUC is commonly referred to as the predictive performance. For example, AUC=0.5 is the minimum level to be considered, while AUC=0 means the test incorrectly classifies all subjects with POCD as non-POCD, and vice versa (Hajian-Tilaki, 2013). In clinical practice, such circumstance is extremely unlikely to happen. Hence, clinicians should evaluate the AUC value and choose the optimum threshold according to clinical threshold.

In healthy individuals, study reported that ERFE concentration to be at median 0.51 ng/ml (Appleby et al., 2020). Compared to our selected threshold value of 0.761 ng/ml, POCD patients has higher ERFE level than healthy person. In other pathological conditions, ERFE was found to be elevated in β-thalassemia, as well as conditions of blood loss or EPO administration (Ganz et al., 2017). In hemodialysis patients, ERFE level was increased and associated with erythropoiesis-stimulating agents (ESA) (Honda et al., 2016). In short, ERFE was reported to be elevated in most erythropoiesis-related diseases, reflecting its function and role in regulating hepcidin.

5.7 Summary

The genes expressed in POCD and CeOx groups were found to be associated with POCD indirectly. In summary, surgery cause genes to be upregulated or downregulated. In POCD groups, the downregulated KIR genes inhibits NK cells activities that may cause neuroinflammation. This later interfere with the neuroprotective effect of NK cells, hence leads to POCD. Likewise, downregulated BTNL3 involved in TNF ERK1/AKT pathway that leads to apoptosis and inflammatory diseases, eventually results in POCD. On the other hand, blood loss that occur intraoperatively may lead to POCD. However, lack of blood triggers the upregulation of ERFE, to increase the ERFE protein that responsible for production of new blood through erythropoiesis. With the increase of ERFE, it maintains the cognitive function postoperatively. In cerebral oximetry patients, neuronal damage genes such as MORN4, CCL23 and GPR37L1 were downregulated, causing a neuroprotective effect to the brain and maintain the patients' cognitive function. In short, the DEGs could involve in the occurrence of POCD or maintain the brain from getting POCD. The explanation was illustrated in Figure 5.2 below.

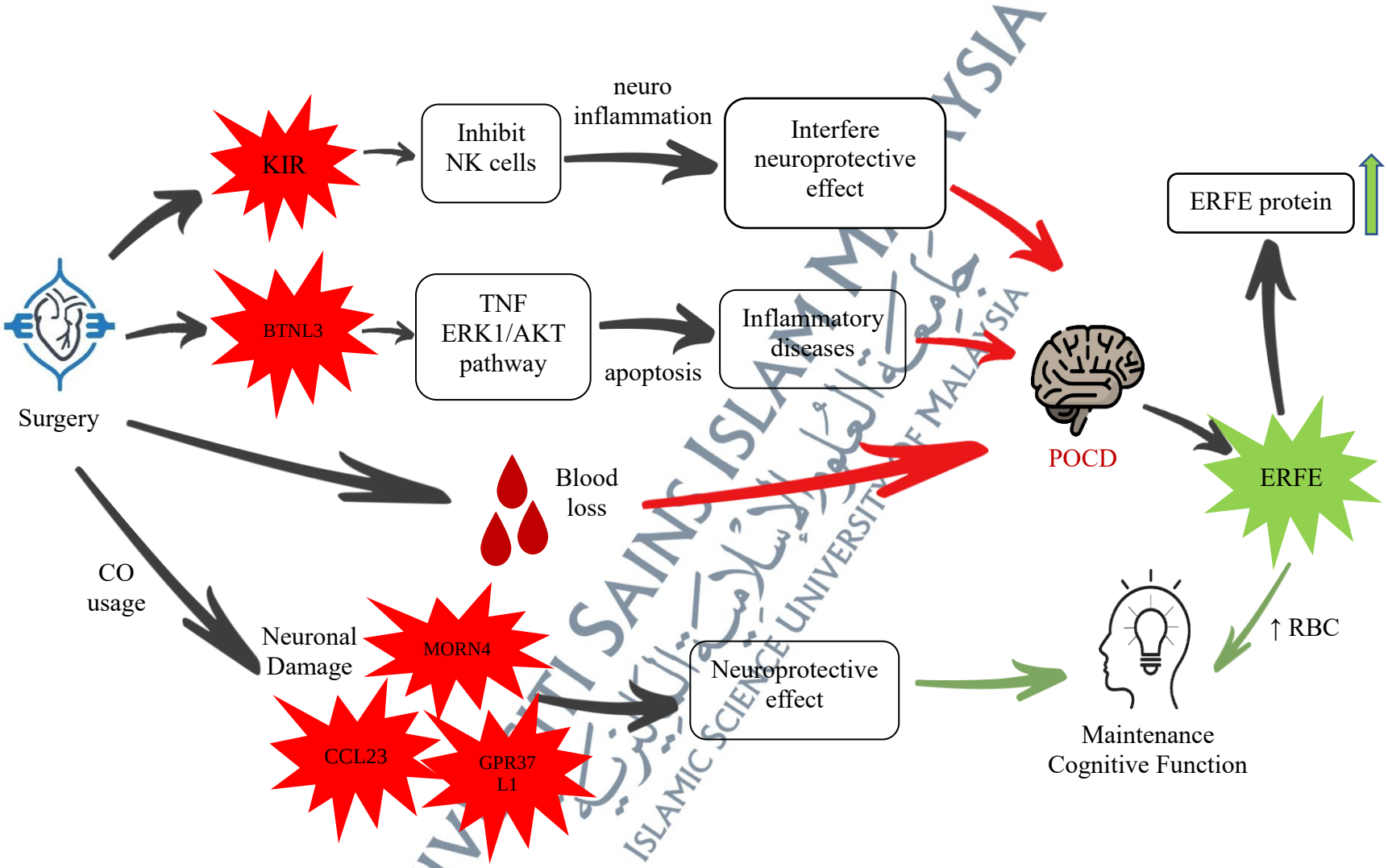

Figure 5.2: Summary of DEGs and their association to POCD occurrence. Genes in red colour was downregulated while in green upregulated.