Week ON12

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SET YOUR WORKING DIRECTORY!

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setwd("C:/Users/1/Dropbox/00 - Working Folder/Teaching/DPH112/2019-2020/Week ON12")
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Announcements

- The two coursework assessments and the final paper have been released. The deadlines are

Assessment	Due Date	Days Till Deadline
Coursework 1	16 May	05
coursework 2	30 May	19
Final Paper	17 June	37

- All the assessments are to be completed INDIVIDUALLY. No collusion is allowed. This means that you cannot discuss this assignment with other students, nor can you share your approach or code.
- The running count for students forwarding errors is as follows:

Student	Items Identified
Yijia Jiang	6
Jing Wang	3
Xinwen Hu	1
Yuxuan Wu	1

Reading

Read and understand the two research papers that are included in ICE for this week.

Introduction

We are taking a break from the discussion on logistic regressio to conduct a special lession. We will finalise the topic of logistic regression next week.

In this sesion, we will examine published research papers that have used multiple linear regression in their analysis. I will discuss two papers. In each paper, we will focus on the methods and presentation of results. You need to know a little bit about the topic of the research, of course, but I will leave that to you to study.

The first paper is by Mutch and colleagues [1]. Dr Mutch led a team that studied whether low carbon dioxide concentrations in the blood (termed *hypocapnia*) is related to the development of changes to the

brain manifesting as mental confusion. These brain changes produce *delirium*. The research team studied this relatipnship in patients undergoing surgery, where delirium is a common occurence after the operation.

The second paper is by Gurholt and colleagues [2]. The paper describes the results of a study that looked at the relationship between two vitamins - folate and vitamin D – on the volume of the part of the skull that encases the brain (termed the *cranium*). They studied patients with severe mental disorders as well as healthy controls.

IMPORTANT: I am using these studies as *models* of currently acceptable ways of presenting statistical information to academic audiences. However, these are only some of the few ways to present your work. There are many, many more ways. You should use your private study time to explore the ways others have done this.

IMPORTANT: It is important that you apply some of these techniques in your own outputs, such as in your coursework for this and other modules, as well as – and probably more importantly – in your final year projects.

Mutch et al.

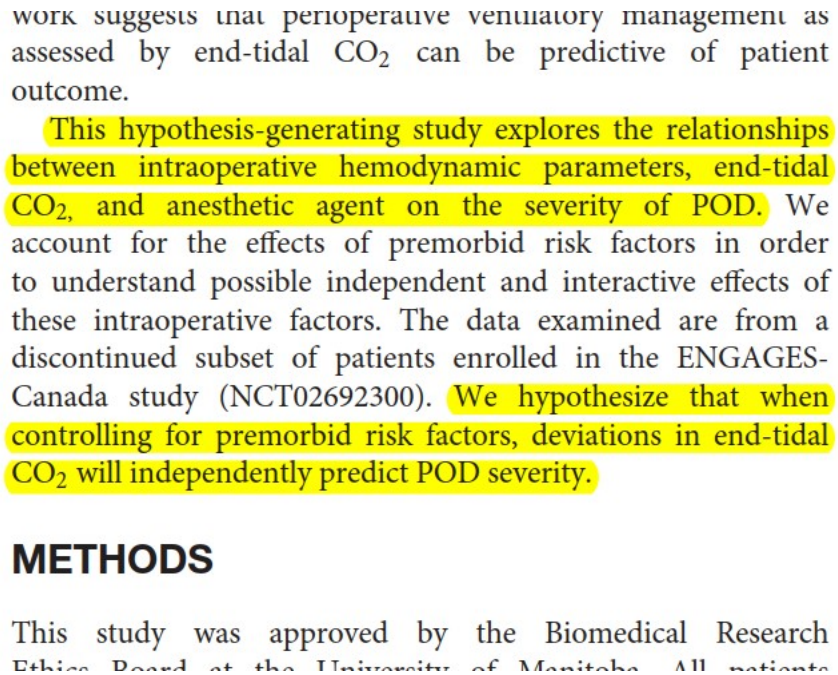
Objectives

The objectives, research questions or hypotheses of the study are the first thing you should understand when reading a paper. In the typical IMRAD paper, you will spot these in two places: the abstract and the last paragraph of the introduction (before the methods section). In Mutch et al., these occur in both places (Figures 1 and 2)

Background: Postoperative delirium (POD) might be associated with anesthetic management, but research has focused on choice or dosage of anesthetic drugs. We examined potential contributions of intraoperative ventilatory and hemodynamic management to POD.

Figure 1: Abstract of Mutch et al. with objective highlighted.

Note how the authors have expanded on the objective in the abstract by stating it in two forms in the final paragraph of the introduction.



work suggests that perioperative ventilatory management as assessed by end-tidal CO_2 can be predictive of patient outcome.

This hypothesis-generating study explores the relationships between intraoperative hemodynamic parameters, end-tidal CO_2, and anesthetic agent on the severity of POD. We account for the effects of premorbid risk factors in order to understand possible independent and interactive effects of these intraoperative factors. The data examined are from a discontinued subset of patients enrolled in the ENGAGES-Canada study (NCT02692300). We hypothesize that when controlling for premorbid risk factors, deviations in end-tidal CO_2 will independently predict POD severity.

METHODS

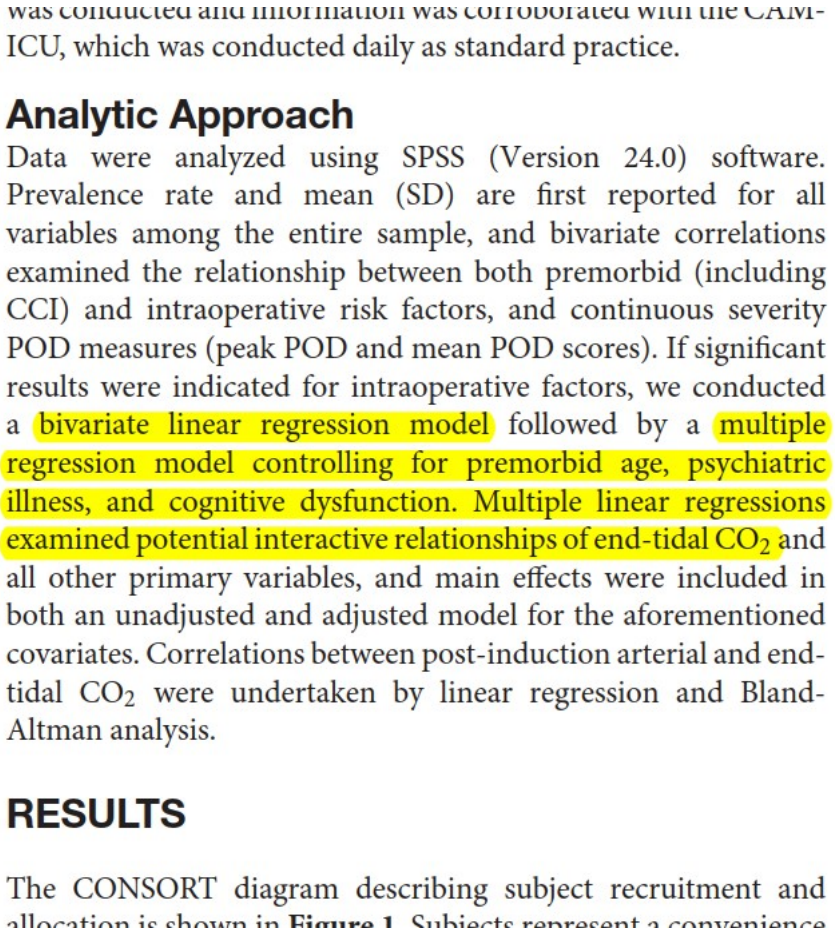
This study was approved by the Biomedical Research Ethics Board at the University of Manitoba. All patients

Figure 2: Paragraph in the introduction of Mutch et al. with objective and hypothesis highlighted.

The hypothesis is very specific. Since they anticipated "controlling for pre-morbit factors", we already know that there will be more than one independent variable. The main independent variable is the carbon dioxide concentration and all others are so-called "nuisance" variables. That is, we measure them in order to control for them. We are really not interested in their results, *per se*.

Methods

In Figure 3, the authors describe their statistical methods. I've highlighted the statements where linear regression methods are mentioned.



was conducted and information was corroborated with the CAM-ICU, which was conducted daily as standard practice.

Analytic Approach

Data were analyzed using SPSS (Version 24.0) software. Prevalence rate and mean (SD) are first reported for all variables among the entire sample, and bivariate correlations examined the relationship between both premorbid (including CCI) and intraoperative risk factors, and continuous severity POD measures (peak POD and mean POD scores). If significant results were indicated for intraoperative factors, we conducted a bivariate linear regression model followed by a multiple regression model controlling for premorbid age, psychiatric illness, and cognitive dysfunction. Multiple linear regressions examined potential interactive relationships of end-tidal CO_2 and all other primary variables, and main effects were included in both an unadjusted and adjusted model for the aforementioned covariates. Correlations between post-induction arterial and end-tidal CO_2 were undertaken by linear regression and Bland-Altman analysis.

RESULTS

The CONSORT diagram describing subject recruitment and allocation is shown in **Figure 1**. Subjects represent a convenience

Figure 3: Statistical analysis in Mutch et al. with regression methods highlighted.

The authors use the term "bivariate linear regression model". This is another term for the simple linear regression model. Thus, thay said that they first conducted a simple linear regression followed by a multiple linear regression. The list of variables that they used is given.

What is not stated is the level of significance and the degree of confidence that they used. It is inappropriate to leave out this important information.

Results

It is usually the case that the first table you will present describes your study population. This is what the authors have done (Figure 4).

TABLE 1 | Premorbid, surgical, and delirium characteristics of the sample and their association to postoperative delirium severity.

		Correlation coefficient	
		POD severity	
	Total sample (n = 101)	Peak score	Mean score
SOCIODEMOGRAPHICS			
Age, years (range = 60-86)	68.7 (6.4)	0.06	**0.21***
Male Sex, *n* (%)	65 (64.4%)	−0.13	−0.12
PREOPERATIVE COGNITIVE FUNCTIONING			
Short Blessed Test, score (range = 0–21)	2.0 (3.1)	**0.29****	0.15
PREOPERATIVE PSYCHIATRIC CHARACTERISTICS			
PHQ Total, score (range = 0–10)	2.4 (2.7)	**0.27****	**0.32****
Alcohol Use, score (range = 0–9)	2.1 (2.2)	−0.01	0.03
PREOPERATIVE PATIENT MORBIDITY			
Charlson Comorbidity Index (range = 0–9)	6.3 (2.5)	−0.04	−0.01
SURGICAL CHARACTERISTICS			
Duration of Anesthesia (min)	209.9 (101.9)	−0.12	−0.10
Mean Arterial Pressure, mm Hg	79.4 (7.9)	−0.01	0.05
End–Tidal CO_2, kPa	4.50 (0.4)	0.05	−0.01
Anesthetic agent (vol%)	1.2 (0.3)	−0.13	−0.19
Anesthetic dose × time (vol% sec)	14994 (8037)	−0.13	−0.17
AUC Blood Pressure (kPa sec)	5080 (2868)	−0.002	0.02
AOC Blood Pressure (kPa sec)	9124 (7193)	−0.04	−0.10
AUC CO_2-0.67kPa	350 (1196)	**0.25***	**0.26***
AOC CO_2-0.67kPa	2453 (3300)	−0.04	0.01
Medications			
Morphine equivalents (range = 2–1509 mg)	69.9 (156.6)	0.14	0.17
Benzodiazepines, presence *n* (%)	32 (33.7%)	0.11	0.03
Ketamine, presence n (%)	20 (21.1%)	0.09	−0.01
Haloperidol, presence n (%)	4 (4.2%)	0.15	0.14
Dimenhydrinate, presence n (%)	25 (26.3%)	0.17	0.10
DELIRIUM			
Preoperative			
History of Previous Delirium, *n* (%)	14 (13.9%)	0.05	0.17
Postoperative			
CAM-S Peak Score	4.2 (2.6)	–	**0.99*****
CAM-S Mean Score	2.5 (1.9)	**0.99*****	–
Days Delirious	0.2 (0.7)	**0.59*****	**0.67*****

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Data are presented as mean (SD) unless otherwise specified and Pearson's correlation coefficient is derived. A point-bi-serial correlation coefficient is reported for dichotomous variables. Perioperative characteristics were evaluated for n = 99 participants. CAM-S, Confusion Assessment Method-Severity Score; AUC, area under the curve; AOC, area over the curve; POD, postoperative delirium. The bold text highlights the statistically different results.

Figure 4: The first table of Mutch et al.

Here is it important to note that they actually have *two* ways to measure the dependent variable (shown by the two columns) – the mean severity of delirium and the peak severity of delirium. This means that they will run simple and multiple linear regressions two times using the two dependent variables one at a time.

In the Mutch et al. paper, you will see that they have provided scatterplots and regression lines with confidence intervals. Based on the degree of confidence that they used, we can deduce that their level of significance was 0.05. Nevertheless, this should have been stated clearly. In each scatterplot in the paper's Figures 3 and 4, the simple linear regression equations are also given.

The regression estimates for the simple and multiple linear regression models are presented in the paper's Table 2 (which I recreate here in Figure 5)

TABLE 2 | Bivariate and multiple linear regressions examining the relationship between significant predictors and delirium severity.

	POD peak score				POD mean score			
	Model 1		Model 2		Model 1		Model 2	
	Beta	***P-Value***	**Beta**	***P-Value***	**Beta**	***P-Value***	**Beta**	***P-Value***
AUC CO_2 0.67 kPa								
Age	0.055	0.593	0.067	0.521	**0.213***	0.038	**0.223***	0.035
Short Blessed Test Score	**0.286****	0.005	**0.338****	0.001	0.149	0.150	**0.220***	0.034
PHQ total score	**0.273****	0.008	0.145	0.159	**0.323****	0.002	0.185	0.075
AUC CO_2	**0.246***	0.020	**0.220***	0.033	**0.261***	0.013	**0.205***	0.048
AOC CO_2 0.67 kPa								
Age	0.055	0.593	0.103	0.334	**0.213***	0.038	**0.250***	0.020
Short Blessed Test Score	**0.286****	0.005	**0.334****	0.002	0.149	0.150	**0.215***	0.044
PHQ total score	**0.273****	0.008	0.184	0.080	**0.323****	0.002	**0.219***	0.038
AOC CO_2	−0.042	0.695	−0.052	0.613	0.007	0.951	−0.034	0.742

*$p < 0.05$, **$p < 0.01$. Standardized betas are reported. Model 1, unadjusted bivariate regressions examining each independent predictor. Model 2, multivariable model including all listed predictors in single model. All covariates entered in models were assessed continuously. CO_2, carbon dioxide; AUC, area under the curve; AOC, area over the curve; PHQ, patient health questionnaire; POD, postoperative delirium. The bold text highlights the statistically different results.

Figure 5: The second table of Mutch et al.

This is actually four tables in one. First, focus on the green highlight. These are the two ways that the dependent variable was measured. Next, focus on the blue highlight. These are the two ways the authors measured the main independent variable. Thus, the four tables in are

- POD peak by AUC carbon dioxide
- POD peak by AUC carbon dioxide
- POD mean by AOC carbon dioxide
- POD mean by AOC carbon dioxide

The authors were very frugal in their design of the table.

Let us focus only on one of the subtables – POD peak by AUC carbon dioxide. Note that there are two models. You will see in the footnote highlighted yellow that Model 1 are the estimates arising from the simple linear regression model and Model 2 are those arising from the multiple linear regression model. This is the typical way to present regression estimates. Finally, note that the intercept is very rarely presented. In this case, this is appropriate because it is physically impossible to have a zero concentration of carbon dioxide in the blood.

Let us list the equations for the all simple linear regression performed for the POD peak by AUC carbon dioxide subtable.

- $POD\ peak\ score = \beta_{01} + 0.055AGE$
- $POD\ peak\ score = \beta_{02} + 0.286BLESSED$
- $POD\ peak\ score = \beta_{03} + 0.273PHQ$
- $POD\ peak\ score = \beta_{04} + 0.246AUC\ CO_2$

Remember, we're really not interested in the first three equations, because they don't feature our main independent variable. However, it is important that we report it, because it gives us information on the nuisance variables.

Finally, the multiple linear regression equation is

$$POD\ peak\ score = \beta_{05} + 0.067AGE + 0.338BLESSED + 0.145PHQ + 0.220AUC\ CO_2$$

You can create equations for the other three subtables, too.

Note that the results of diagnostic tests are not presented. In general, published articles leave out this information. Howevere, it does not mean that diagnostic testing was not performed.

Gurholt et al.

Objectives

The objectives for the study by Gurholt and colleagues appear in the paper in the expected areas (Figures 6 and 7).

Vitamin D and folate deficiency are considered risk factors for schizophrenia and bipolar disorders, but it is unknown how vitamin D and folate influence the growing brain, cranium or the clinical phenotype. Serum vitamin D and folate levels are in part genetically regulated. We investigated whether adult vitamin D and folate levels are associated with the intracranial volume (ICV) under the hypothesis that developmental vitamin D or folate levels influence neurodevelopment and that current levels are associated with ICV. Ninety patients with severe mental disorders and 91 healthy controls underwent 3T magnetic resonance imaging and serum sampling. Multiple linear regression was used to assess the contribution of serum vitamin D, folate and patient-control status on ICV. We show that vitamin D levels were within lower range for patients and controls (48.8 ± 22.1 nmol/l and 53.4 ± 20.0 nmol/l, respectively). A significant positive association was found between vitamin D and ICV ($p = 0.003$, $r = 0.22$), folate was trend-significantly associated with ICV. Folate and vitamin D were significantly associated ($p = 0.0001$, $r = 0.28$). There were nonsignificant patient-control differences and no interaction effects. The results suggest that Vitamin D is associated with ICV as detected in the adult. Further studies are warranted for replication and to investigate possible mechanisms and genetic associations.

Figure 6: Abstract of Gurholt et al. with objective highlighted.

tial to influence brain structures, including ICV as measured in adults, in addition to nutritional factors.

Folate and vitamin D levels have both been implicated as risk factors for developing schizophrenia and bipolar spectrum disorders, as well as in brain and bone development. Vitamin D is essential for cranial development in early life, and vitamin D and folate insufficiency could be associated with the smaller ICV as reported in schizophrenia. The aim of the present study was to investigate a putative association between current vitamin D and S-folate levels with ICV measured from magnetic resonance imaging (MRI) scans of adult patients with severe mental disorders and healthy controls. We expected to find vitamin D and S-folate to be associated with ICV both in the patients with severe mental disorders and the healthy controls, but with a stronger association in the patients. We expected vitamin D to have a stronger association with ICV than S-folate.

Methods and Materials

Study population. The subject sample consisted of 90 patients with severe mental disorders (*Schizophrenia*

Figure 7: Paragraph in the introduction of Gurholt et al. with objective and hypothesis highlighted.

Methods

The statistical methods, shown in Figure 8, are quite well described. Similar to the previous example, there are two main independent variables – serum vitamin D (S-25(OH)D) and serum folate (S-folate). The authors defined three regression models. Model 1 is the linear regression of intracranial volume (ICV) on the first independent variable, S-25(OH)D. Model 2 is the linear regression of ICV on S-folate. The final model, Model 3, is the linear regression of ICV on both S-25(OH)D and S-folate at the same time. All models adjust for a numnber of nuisance variables. Therefore, unlike the previous example, all models in Gurholt are multiple linear regression models.

Statistical method. The demographic variables of patients and controls were compared using two-sample t-test for continuous variables with a normal distribution and the two-sided Wilcoxon rank sum test was used for continuous variables with a non-normal distribution. The distributions were considered normal if they passed the Shapiro-Wilk's normality test[54] and their histograms were normal from visual evaluation. For categorical variables the χ^2 -test was used.

Multiple linear regression was used to investigate the association between S-25(OH)D, S-folate and ICV, and between S-25(OH)D and S-folate, using the R statistical software's *lm* function. The association between S-25(OH)D, S-folate and ICV was investigated both individually (Model 1 and Model 2, respectively) and together (Model 3) while also adjusting for group (patient-control status), age, sex, weight, height, ethnicity (Caucasian or non-Caucasian origin) and assessment season (winter: November-April, summer: May-October). Post hoc analysis were performed in post hoc Model A and Model B to investigate the association between S-25(OH)D and S-folate, firstly while adjusting for group, age, sex, ethnicity and assessment season, and secondly while also adjusting for weight and height. In the supplemental information, additional post hoc analyses explored the possibility of group and season specific contributions on the above models. Group, ethnicity and assessment season are binary variables.

The regression results are expressed as standardized β coefficients, 95% confidence interval and p-values. The effect size is reported as Cohen's d for categorical variables and the partial correlation coefficient, r, for continuous variables[55]. F statistics was used to assess the contribution of additional covariates[56].

Figure 8: Statistical analysis in Gurholt et al. with regression methods highlighted.

Pay attention to the sentence highlighted in blue. Here, the authors do state the level of significance that they used. Importantly, they also state that all model estimates are standardised. (Review the materials in Week 10 if you need to.) This means that all results are not in volumetric units (e.g., mL) but in unitless values of standard deviations units.

IMPORTANT: The authors state that the R script that they used is available if you write to the first author. I suggest that you do so. This will give you an idea of the way an experienced analyst structures his code.

Results

Again, similar to the previous example, Table 1 in the original paper presents descriptions of the study population. You should learn to practice this.

The main results for the regression model is given in Table 2 in the original paper (which I recreate here as Figure 9).

	Dependent variable: ICV								
	Model 1			Model 2			Model 3		
Covariates	β	95% CI	p-value	β	95% CI	p-value	β	95% CI	p-value
S-25(OH)D	0.2	(0.1, 0.3)	**0.003**				0.2	(0.04, 0.3)	**0.011**
S-folate				0.1	(−0.002, 0.2)	0.053	0.1	(−0.1, 0.2)	0.233
Group[a]	0.01	(−0.2, 0.3)	0.92	−0.02	(−0.3, 0.2)	0.856	−0.01	(−0.3, 0.2)	0.929
Age	−0.001	(−0.1, 0.1)	0.99	−0.003	(−0.1, 0.1)	0.968	−0.02	(−0.1, 0.1)	0.799
Sex[b]	0.8	(0.5, 1.2)	**6.4e-06**	0.8	(0.5, 1.2)	**1.2e-05**	0.8	(0.5, 1.2)	**5.1e-06**
A. season[c]	−0.04	(−0.3, 0.2)	0.72	0.1	(−0.2, 0.3)	0.688	−0.03	(−0.3, 0.2)	0.794
Weight	−0.2	(−0.4, −0.1)	**0.004**	−0.2	(−0.4, −0.1)	**0.007**	−0.2	(−0.4, −0.1)	**0.007**
Height	0.4	(0.2, 0.6)	**4.5e-05**	0.4	(0.2, 0.6)	**5.5e-05**	0.4	(0.2, 0.6)	**5.4e-05**
Ethnicity[d]	−0.4	(−0.8, −0.02)	**0.041**	−0.5	(−0.9, −0.1)	**0.017**	−0.4	(−0.8, 0.01)	0.054

Table 2. The association between S-25(OH)D, S-folate and ICV for patients and controls. *Notes:* Regression model with N = 181 participants (90 patients, 91 controls) and standardized continuous covariates. Model 1: S-25(OH)D on ICV, Model 2: S-folate on ICV, Model 3: S-25(OH)D and S-folate on ICV. Significance threshold $p < 0.05$ indicated in bold. Group: Patient-Control status, CI: Confidence Interval, winter: November-April, summer: May-October, Ethnicity: Caucasian or non-Caucasian origin, A. season: Assessment season. [a]Reference group: controls. [b]Reference sex: women. [c]Reference season: winter. [d]Reference ethnicity: Caucasian.

Figure 9: The regression results of Gurholt et al.

Model 1, which includes S-25(OH)D alone, does not show a result for S-folate. Model 2, which includes S-folate alone, does not include results for S-25(OH)D. Finally, Model 3 includes both S-25(OH)D and S-folate. Note, too, how all other variables are included in these results.

Now, pay attention to the footnote. Notice how the authors have stated what the reference categories are for the categorical independent variables that were included in the model. For example, footnote c states that the reference cagetory for `sex` is female.

Can you try to produce the regression equation for each of the three models?

The major omission in Gurholt et al. was that they did not present scatterplots. This is very poor practice. It shows that even work published in some of the best journals may contain serious errors.

References

1. Mutch WAC, El-Gabalawy R, Girling L, Kilborn K and Jacobsohn E. End-tidal hypocapnia under anesthesia predicts postoperative delirium. *Front Neurol* 2018;9:678.

2. Gurholt TP, Osnes K, Nerhus M, Jorgensen KN, Lonning V, Berg A, Andreassen OA, Melle I, Agartz I. Vitamin D, folate and the intracranial volume in schizophrenia and bipolar disorder and healthy controls. *Sci Reports* 2018;8:10817.

THE END