Supplementary Material

Concordance of Alzheimer's Disease-Related Biomarkers Between Intraventricular and Lumbar Cerebrospinal Fluid in Idiopathic Normal Pressure Hydrocephalus

Methods

In vitro experiment Protocol 1

The protocol of conducted experiment was divided in 5 phases (here referred 1-5). In addition, the preparation of samples and analysis phases were executed. The Preparation included approximately 45 ml of cerebrospinal fluid (CSF). The samples were collected by lumbar puncture, centrifuged, aliquoted, and frozen in -80°C freezer in the 5 ml polypropene tubes (Sarstedt) and further thawed prior the use. The CSF was collected from suspected idiopathic normal pressure hydrocephalus (iNPH) patients that did not undergo the shunt surgery. The thawed samples were mixed into three 15 ml polypropene tubes (Fisherbrand) to acquire three CSF mixtures (here referred mixture 1, 2, and 3) with diverging compositions. Each mixture was mixed by turning upside-down 20 times. The analysis of the all samples produced was made similarly to the protocol of 97 patients cohort presented in the methods sections. Briefly, amyloid-β 1-42, total tau, and phosphorylated tau 181 concentrations were measured by fully automated Elecsys immunoassays (Roche Diagnostics GmbH, Penzberg, Germany). All samples had one additional freeze-thaw cycle prior the analysis. The full protocol resulted 30 0.5 ml samples for analysis.

Phase 1 included the obtainment of baseline samples. In all, two baseline samples were obtained from each mixture by pipetting 0.5 ml of CSF into the 0.5 ml sampling tubes (Sarstedt).

Phase 2 simulated the effects of the CSF shunt intracranial part to CSF biomarker concentrations. First, the CSF shunt catheter was rinsed with isotonic natriumchlorid solution. After the rinse, the remaining solution inside the catheter was emptied by micropipette. Then catheter head was inserted into the CSF mixture and waste sample aspirated and ejected away. Further, 1 ml sample was aspirated by micropipette and ejected to the 13 ml polypropene (Sarstedt) tube. The tube was then lightly circulated. Thereafter, two 0.5 ml samples were obtained by micropipette to two 0.5 ml sampling tubes (Sarstedt). The same protocol was then implemented for the mixture 2 and 3.

In phase 3, the whole inflow parts (catheter and shunt valve) of CSF shunt (PS Medical Strata II) were included. The phase 3 mimics our protocol used in V-CSF sample collection of 41-patient cohort. First the CSF shunt was rinsed with isotonic natriumchlorid solution. After the rinse, the remaining solution inside the shunt was emptied by micropipette. Next the catheter was inserted into the mixture 1. The CSF shunt system was then fulfilled with CSF mixture and 2 ml waste sample was obtained by using the 26 gauge x 5/8" needle (BD Microlance 3, Becton, Dickinson and Company Ltd, Drogheda, Ireland), 3-way stopcock with 10 cm tubing (Discofix C 10 cm, Braun Medical AG, Escholzmatt, Switzerland) and 20 ml syringe (BD Discardit II, Becton Dickinson S.A., Fraga Spain). Further, the waste sample was ejected, and 5 ml sample was aspirated into the syringe. The obtained sample was ejected to 15 ml polypropene tube (Sarstedt), and the tube was lightly circulated. Thereafter, two 0.5 ml samples were pipetted into two 0.5 ml sampling tubes. The same protocol was then implemented for the mixture 2 and 3.

Phase 4 was like the phase 3, except the CSF samples were obtained directly from the CSF mixtures without the CSF shunt system in-between. In addition, there were no waste sample collected and the obtained sample was 2 ml per mixture. This phase was implemented to differentiate the effect of the needle, 3-way stopcock with 10 cm tubing and syringe to the CSF biomarker concentrations. In all, 3 x 2 x 0.5 ml of samples were collected.

Phase 5 included the endpoint samples. Two 0.5 ml samples were pipetted from each CSF mixture to the 0.5 ml sampling tubes (Sarstedt).

In vitro experiment protocol 2

The second protocol we conducted had in 3 phases (here referred 1-3). The preparation, handling and analysis of samples were corresponding to the protocol 1. Here we used approximately 165 ml of CSF that was divided into three 50 ml polypropene tubes (Fisherbrand) (Mixtures 1, 2, and 3). In all, this protocol provided 21 0.5 ml samples.

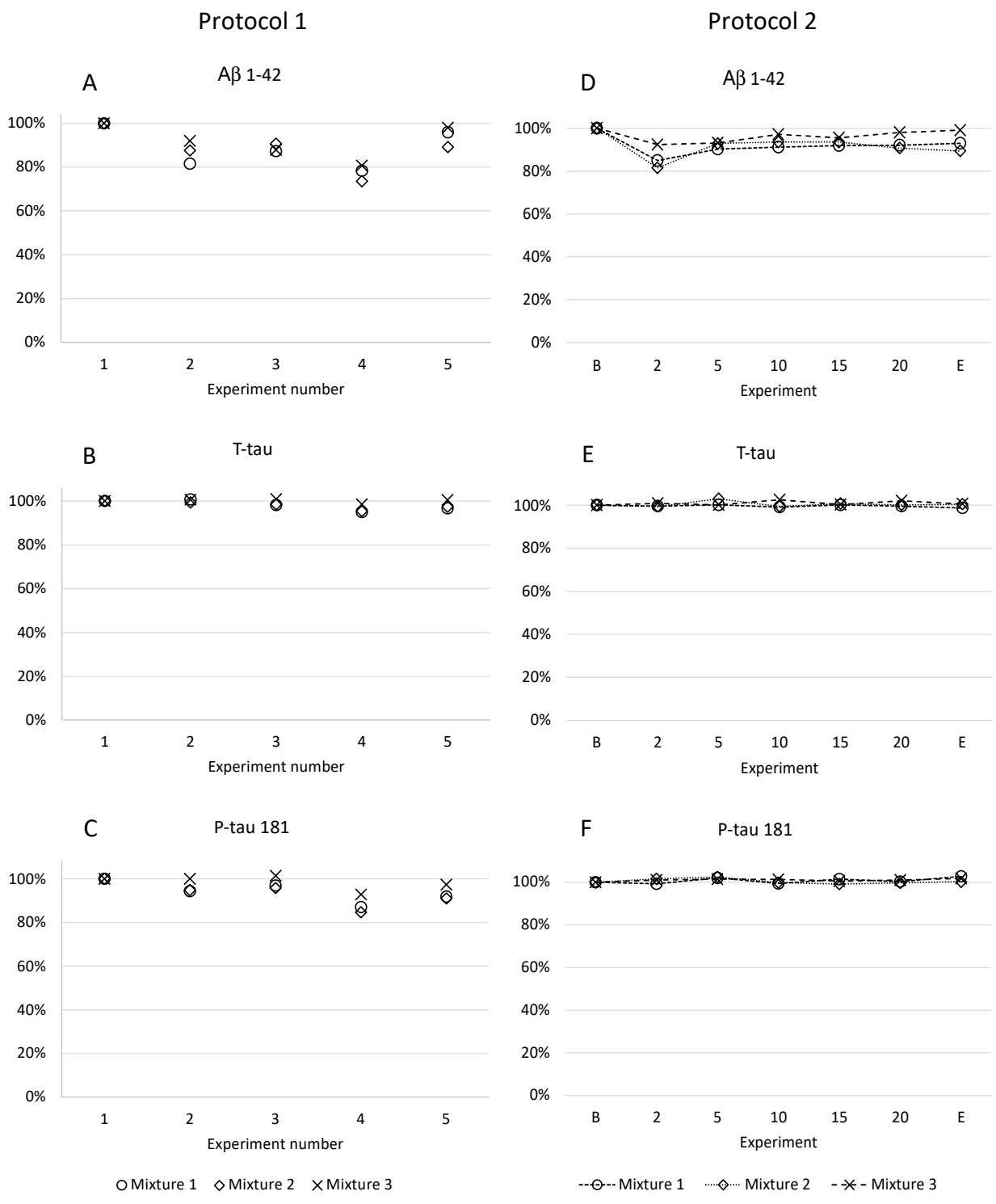
In phase 1, we obtained baseline samples by micropipette. Three 0.5 ml samples were obtained into the 0.5 ml sampling tubes (Sarstedt).

Phase 2 included various sample sizes collected by 3-way stopcock with 10 cm tubing (Discofix C 10 cm, Braun Medical AG, Escholzmatt, Switzerland) and 20 ml syringe (BD Discardit II, Becton Dickinson S.A., Fraga Spain). The quantities of 2, 5, 10, 15, and 20 ml of CSF were aspirated one by one, and each volume had individual by 3-way stopcock with 10 cm tubing

and syringe used. The aspirates were further ejected to 15 ml polypropene tubes (Fisherbrand). The 3-way stopcock with 10 cm tubing was removed from to syringe prior the ejection of samples. Thereafter, the tubes were lightly circulated, and 0.5 ml samples were pipetted from each 15 ml tube. The same protocol was then implemented for the mixture 2 and 3.

In phase 5, 0.5 ml endpoint samples from each mixture were collected and transferred to 0.5 ml sampling tubes (Sarstedt) by micropipette.

Supplementary Figure 1. *In vitro* experiment: percentual change of $A\beta_{42}$, T-tau, and P-tau_{181} from the baseline concentration. A-C are presenting protocol 1 and D-F protocol 2, $A\beta_{42}$, T-tau, and P-tau_{181} mean percentual changes from baseline of our *in vitro* experiment. The experiment numbers are presenting different phases of the protocol 1. Number 1 stands for baseline. In phase 2, sample is collected by micropipette, through the silicone catheter of the CSF shunt. In phase 3, sample is collected by aspirating the sample from inflow catheter through shunt reservoir by the combination of needle, 3-way stopcock, and syringe. For phase 4 sample is obtained by needle, 3-way stopcock, and syringe directly from the CSF mixture used in the experiment. Number 5 is indicating endpoint samples obtained. In protocol 2 experiment section classifies different sections similarly. Baseline and endpoint (B and E) samples are obtained by micropipette from the CSF mixture used. Further, the numbers presented (2, 5, 10, 15, and 20), illustrate different sample sizes (ml of CSF) that were obtained by the combination of 3-way stopcock and syringe. The different mixtures used in both protocols are presented with individual symbols (circle, square, and cross). $A\beta_{42}$, amyloid-β 1-42; T-tau, total tau protein; P-Tau_{181}, hyperphosphorylated tau at threonine 181; CSF, cerebrospinal fluid.


Protocol 1
Protocol 2
A
Aβ 1-42
D
Aβ 1-42
100%
80%
60%
40%
20%
0%
1
2
3
4
5
Experiment number
B
2
5
10
15
20
E
Experiment
B
T-tau
E
T-tau
C
P-tau 181
F
P-tau 181
Mixture 1
Mixture 2
Mixture 3

Supplementary Table 1. Mean percentual change from baseline in protocol 1

Phase	$A\beta_{42}$	T-tau	P-tau
Baseline	100 %	100 %	100 %
2	87.1 %	100.2 %	96.4 %
3	88.6 %	99.2 %	98.0 %
4	77.6 %	96.3 %	88.3 %
Endpoint	94.3 %	98.2 %	93.4 %

The *in-vitro* experiment protocol 1 mean percentual changes from baseline, presented for $A\beta_{42}$, T-tau, and P-tau_{181}. Phase is indicating the different sections used for experiment. Phase 2 sample is collected by micropipette, through the silicone catheter of the CSF shunt. Phase 3 sample is collected by aspirating the sample from inflow catheter through shunt reservoir by the combination of needle, 3-way stopcock, and syringe. Phase 4 sample is obtained by needle, 3-way stopcock, and syringe directly from the CSF mixture used in the experiment. Endpoint and baseline samples are obtained directly using the micropipette. $A\beta_{42}$, amyloid-β 1-42; T-tau, total tau protein; P-Tau_{181}, hyperphosphorylated tau at threonine 181; CSF, cerebrospinal fluid.

Supplementary Table 2. Mean percentual change from baseline in protocol 2

	$A\beta_{42}$	**T-tau**	**P-tau181**
Baseline	100 %	100 %	100 %
2 ml	86.3 %	100.0 %	100.6 %
5 ml	92.1 %	101.1 %	102.0 %
10 ml	93.9 %	100.4 %	100.2 %
15 ml	93.7 %	100.5 %	100.4 %
20 ml	93.7 %	100.6 %	100.3 %
Endpoint	93.8 %	100.0 %	101.6 %

The *in vitro* experiment protocol 2 mean percentual changes from baseline, presented for $A\beta_{42}$, T-tau, and P-tau_{181}. Baseline and endpoint samples are obtained by micropipette from the CSF mixture used. Different sample sizes were obtained by the combination of 3-way stopcock and syringe. $A\beta_{42}$, amyloid-β 1-42; T-tau, total tau protein; P-Tau_{181}, hyperphosphorylated tau at threonine 181; CSF, cerebrospinal fluid.

Supplementary Table 3. Biomarker concentrations of the *in vitro* experiment protocol 1 (pg/ml)

		$A\beta_{42}$			T-tau			P-tau181		
Phase	**Sample**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
1	**1**	721.5	1189	1028	178.3	218.0	214.4	11.1	18.7	15.4
1	**2**	752.6	1168	1093	171.6	228.5	210.1	11.2	19.6	15.8
2	**1**	589.1	1056	969.5	179.3	221.7	216.6	10.6	18.1	15.8
2	**2**	614.1	1009	981.2	173.0	222.1	210.3	10.4	18.2	15.4
3	**1**	642.9	1077	949.6	171.0	219.5	217.7	11.0	18.2	15.8
3	**2**	643.6	1060	915.1	172.8	220.0	210.7	10.6	18.5	15.7
4	**1**	551.6	862.8	863.8	167.7	210.0	209.0	9.9	15.8	14.6
4	**2**	603.8	871.5	848.1	164.5	215.6	209.2	9.6	16.6	14.3
5	**1**	681.4	1113	1041	172.0	218.0	209.6	10.3	17.3	15.0
5	**2**	731.4	987.8	1038	166.3	216.5	217.0	10.2	17.6	15.3

The *in vitro* experiment protocol 1 concentrations (pg/ml) of $A\beta_{42}$, T-tau, and P-tau_{181}. The phase is indicating the different sections used for experiment. Phase 2 sample is collected by micropipette, through the silicone catheter of the CSF shunt. Phase 3 sample is collected by aspirating the sample from inflow catheter through shunt reservoir by the combination of needle, 3-way stopcock, and syringe. Phase 4 sample is obtained by needle, 3-way stopcock, and syringe directly from the CSF mixture used in the experiment. Endpoint (phase 5) and baseline (phase 1) samples are obtained directly using the micropipette. The different mixtures of CSF are indicated with the number below the biomarkers (Mixtures 1, 2, and 3). $A\beta_{42}$, amyloid-β 1-42; T-tau, total tau protein; P-Tau_{181}, hyperphosphorylated tau at threonine 181; CSF, cerebrospinal fluid.

Supplementary Table 4. Biomarker concentrations of the *in vitro* experiment protocol 2 (pg/ml)

	$A\beta_{42}$			**T-tau**			**P-tau181**		
Mixture	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Baseline	667.0	727.8	481.2	213.9	206.2	214.1	18.6	14.6	16.0
2 ml	566.2	593.4	444.6	213.1	205.0	216.1	18.5	14.8	16.2
5 ml	602.1	676.2	448.2	214.4	212.3	214.5	19.0	15.0	16.2
10 ml	607.8	681.3	467.3	212.2	205.2	219.7	18.5	14.6	16.2
15 ml	612.9	680.7	459.8	214.3	207.9	214.9	18.9	14.5	16.1
20 ml	614.6	660.1	472.1	213.0	206.6	218.5	18.7	14.5	16.2
Endpoint	620.2	650.6	477.2	211.3	207.2	215.6	19.1	14.6	16.3

The *in vitro* experiment protocol 2 concentrations (pg/ml) of $A\beta_{42}$, T-tau, and P-tau_{181}. Baseline and endpoint samples are obtained by micropipette from the CSF mixture used. Different sample sizes were obtained by the combination of 3-way stopcock and syringe. $A\beta_{42}$, amyloid-β 1-42; T-tau, total tau protein; P-Tau_{181}, hyperphosphorylated tau at threonine 181; CSF, cerebrospinal fluid.