Supplementary Material

Supplementary Table 1 Variables linked to cold agglutinins

| Patient no. | Sex | CA test | CA titer | UCT score | Hemoglobin level (g/L) | Erythrocyte count (10^12/L) | Monocyte count (10^9/L) | Thrombocyte count (10^9/L) | Mean daily Temp. (°C) |
|-------------|-----|---------|----------|-----------|------------------------|-----------------------------|--------------------------|--------------------------|----------------------|
| 1           | F   | Neg     | 0        | 13        | 141                    | 4.61                        | 1.01                     | 340                      | 0                    |
| 2           | M   | Pos     | 1        | 5         | 137                    | 4.44                        | 0.38                     | 271                      | 17                   |
| 3           | F   | Pos     | 2        | 3         | 132                    | 4.51                        | 0.35                     | 468                      | 25                   |
| 4           | M   | Neg     | 0        | 16        | 149                    | 4.81                        | 0.57                     | 236                      | 18                   |
| 5           | F   | Pos     | 4        | 3         | 146                    | 4.85                        | 0.50                     | 209                      | 17                   |
| 6           | F   | Pos     | 4        | 7         | 140                    | 4.24                        | 0.57                     | 330                      | 13                   |
| 7           | M   | Neg     | 0        | 11        | 147                    | 4.66                        | 0.61                     | 257                      | 15                   |
| 8           | F   | Pos     | 1        | 12        | 138                    | 4.58                        | 0.37                     | 275                      | 21                   |
| 9           | F   | Neg     | 0        | 6         | 154                    | 4.80                        | 0.60                     | 266                      | 4                    |
| 10          | M   | Neg     | 0        | 16        | 159                    | 4.92                        | 0.91                     | 237                      | 16                   |
| 11          | F   | Pos     | 2        | 12        | 146                    | 4.74                        | 0.64                     | 318                      | 21                   |
| 12          | F   | Pos     | 4        | 14        | 139                    | 4.32                        | 0.41                     | 203                      | 22                   |
| 13          | M   | Neg     | 0        | 9         | 154                    | 5.22                        | 0.50                     | 371                      | 21                   |
| 14          | F   | Pos     | 1        | 7         | 130                    | 4.02                        | 0.05                     | 301                      | 18                   |
| 15          | F   | Neg     | 0        | 6         | 130                    | 4.19                        | 0.79                     | 272                      | 2                    |
| 16          | M   | Pos     | 2        | 13        | 143                    | 4.99                        | 0.71                     | 365                      | 21                   |
| 17          | M   | Neg     | 0        | 15        | 150                    | 5.13                        | 0.61                     | 282                      | 6                    |
| 18          | M   | Neg     | 0        | 16        | 159                    | 5.43                        | 0.56                     | 259                      | 17                   |
| 19          | F   | Neg     | 0        | 15        | 135                    | 4.37                        | 0.69                     | 269                      | 15                   |
| 20          | F   | Pos     | 2        | 9         | 134                    | 4.17                        | 0.58                     | 292                      | 13                   |
| 21          | F   | Pos     | 4        | 11        | 120                    | 4.27                        | 0.20                     | 274                      | 22                   |
| 22          | F   | Neg     | 0        | 16        | 145                    | 4.42                        | 0.69                     | 222                      | 2                    |
| 23          | F   | Pos     | 16       | 12        | 145                    | 5.06                        | 0.37                     | 335                      | 10                   |
| 24          | F   | Neg     | 0        | 14        | 151                    | 4.73                        | 0.53                     | 170                      | 2                    |
| 25          | F   | Neg     | 0        | 10        | 146                    | 4.76                        | 0.38                     | 197                      | 10                   |
| 26          | F   | Neg     | 0        | 16        | 142                    | 4.43                        | 0.53                     | 220                      | 13                   |
| 27          | M   | Neg     | 0        | 16        | 162                    | 6.02                        | 0.53                     | 150                      | 8                    |
| 28          | F   | Pos     | 1        | 12        | 134                    | 4.69                        | 0.40                     | 285                      | 0                    |
| 29          | F   | Pos     | 2        | 7         | 123                    | 4.44                        | 0.33                     | 248                      | 10                   |
| 30          | M   | Neg     | 0        | 13        | 146                    | 4.98                        | 0.35                     | 208                      | 13                   |
| 31          | F   | Pos     | 2        | 10        | 128                    | 3.98                        | 0.33                     | 228                      | 13                   |
| 32          | M   | Neg     | 0        | 13        | 149                    | 4.90                        | 0.41                     | 222                      | 7                    |
| 33          | M   | Neg     | 0        | 5         | 161                    | 5.27                        | 0.47                     | 271                      | 5                    |
| 34          | F   | Pos     | 1        | 11        | 137                    | 4.22                        | 0.30                     | 222                      | 7                    |
| 35          | F   | Neg     | 0        | 4         | 139                    | 4.69                        | 0.52                     | 233                      | 2                    |

Cold agglutinin test performed at 4°C. The numbers are inverse of the maximum serum dilution at which agglutination can be seen (i.e., CA titer 4 = agglutination at 1:4 dilution). Mean daily temperature on the day when blood was drawn was obtained from the national weather service website (http://meteo.arso.gov.si/met/en/). Abbreviations: CA, cold agglutinin; F, female; M, male; Neg, negative; Pos, positive; Temp., temperature; UCT, Urticaria Control Test.
## Supplementary Table 2 Variables linked to cryoglobulins

| Patient no. | CG test | CG conc. (mg/L) | CG Ig isotype(s) | ColdU duration (months) | Lymphocyte count (10^9/L) | Basal serum tryptase level (ng/mL) |
|-------------|---------|-----------------|------------------|--------------------------|--------------------------|-----------------------------------|
| 1           | Pos     | 137             | IgG, IgM, IgA     | 70                       | 2.13                     | 3.66                              |
| 2           | Neg     | —               | —                | 14                       | 1.55                     | 3.24                              |
| 3           | Neg     | —               | —                | 24                       | 2.58                     | 3.41                              |
| 4           | Neg     | —               | —                | 60                       | 2.49                     | 11.60                             |
| 5           | Neg     | —               | —                | 336                      | 1.39                     | 8.15                              |
| 6           | Neg     | —               | —                | 9                        | 1.22                     | 15.90                             |
| 7           | Neg     | —               | —                | 4                        | 1.94                     | 5.34                              |
| 8           | Neg     | —               | —                | 50                       | 1.63                     | 5.32                              |
| 9           | Pos     | 189             | IgG              | 169                      | 2.89                     | 7.57                              |
| 10          | Neg     | —               | —                | 120                      | 1.52                     | 6.19                              |
| 11          | Neg     | —               | —                | 228                      | 2.49                     | 23.00                             |
| 12          | Neg     | —               | —                | 156                      | 2.68                     | 3.57                              |
| 13          | Neg     | —               | —                | 60                       | 2.44                     | ND                                |
| 14          | Neg     | —               | —                | 99                       | 1.94                     | 5.35                              |
| 15          | Pos     | 124             | IgG, IgM         | 24                       | 1.88                     | 3.83                              |
| 16          | Neg     | —               | —                | 24                       | 1.40                     | 5.50                              |
| 17          | Neg     | —               | —                | 240                      | 1.99                     | 7.79                              |
| 18          | Neg     | —               | —                | 10                       | 1.34                     | 4.44                              |
| 19          | Pos     | 184             | IgG, IgM         | 252                      | 2.67                     | 13.60                             |
| 20          | Neg     | —               | —                | 276                      | 1.70                     | 5.49                              |
| 21          | Neg     | —               | —                | 72                       | 1.20                     | 3.96                              |
| 22          | Neg     | —               | —                | 120                      | 2.61                     | 6.37                              |
| 23          | Pos     | 153             | IgG, IgM, IgA    | 216                      | 2.62                     | 5.55                              |
| 24          | Neg     | —               | —                | 384                      | 1.80                     | 7.41                              |
| 25          | Neg     | —               | —                | 2                        | 1.13                     | 6.00                              |
| 26          | Neg     | —               | —                | 22                       | 2.06                     | 4.55                              |
| 27          | Neg     | —               | —                | 15                       | 2.01                     | 12.70                             |
| 28          | Pos     | 187             | IgG, IgM         | 84                       | 1.72                     | 6.09                              |
| 29          | Neg     | —               | —                | 8                        | 1.75                     | 3.05                              |
| 30          | Pos     | 106             | IgG, IgM         | 60                       | 1.22                     | 2.92                              |
| 31          | Neg     | —               | —                | 42                       | 1.95                     | 5.10                              |
| 32          | ND      | —               | —                | 8                        | 1.47                     | 2.90                              |
| 33          | Pos     | 138             | IgG, IgM         | 72                       | 1.49                     | 6.06                              |
| 34          | ND      | —               | —                | 22                       | 2.17                     | 5.33                              |
| 35          | Pos     | 150             | IgG              | 11                       | 2.12                     | 14.00                             |

**Abbreviations:** conc., concentration; CG(s), cryoglobulin(s); CRP, C-reactive protein; Ig, immunoglobulin; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; Neg, negative; ND, not done/determined; Pos, positive.
## Supplementary Table 3

Characteristics of patients with a positive vs. negative cryoglobulin test

| Parameter                              | Total  | CG test | Positive | $P$ value  |
|----------------------------------------|--------|---------|----------|------------|
|                                       | $N = 33$ | $N = 24$ | $N = 9$ | (positive vs. negative) |
| Female sex                             | 22 (67) | 15 (63) | 7 (78)  | 0.681      |
| Triggers                               |        |         |          |            |
| Cold ambient air                       | 27 (82) | 20 (83) | 7 (78)  | 1.000      |
| Immersion in $< 25^\circ$C water       | 18 (55) | 13 (54) | 5 (56)  | 0.627      |
| Higher summer humidity levels          | 15 (46) | 11 (46) | 4 (44)  | 1.000      |
| Ingestion of cold foods/drinks         | 18 (55) | 14 (58) | 4 (44)  | 0.697      |
| UCT score                              | 12 (7–15) | 12 (8–16) | 12 (6–13) | 0.310      |
| CST results                            |        |         |          |            |
| Positive ice cube test                 | 20 (61) | 16 (67) | 4 (44)  | 0.425      |
| Positive TempTest®                    | 12 (36) | 11 (46) | 1 (11)  | 0.107      |
| CSTT (s)                               | 240 (30–300); $N = 18$ | 150 (30–300); $N = 14$ | 300 (165–300); $N = 4$ | 0.182      |
| CTT (°C)                               | 19 (15–25); $N = 12$ | 21 (16–25); $N = 11$ | 14; $N = 1$ | 0.242      |
| Laboratory findings                    |        |         |          |            |
| Erythrocyte count (10$^{12}$/L)        | 4.7 ± 0.4 | 4.7 ± 0.5 | 4.7 ± 0.3 | 0.685      |
| Hemoglobin concentration (g/L)         | 142.6 ± 10.6 | 142.5 ± 11.0 | 142.8 ± 10.0 | 0.948      |
| Hematocrit level (%)                   | 41.7 ± 3.4 | 41.6 ± 3.4 | 41.9 ± 3.5 | 0.828      |
| Thrombocyte count (10$^{9}$/L)         | 268.6 ± 63.2 | 266.0 ± 70.1 | 275.4 ± 42.3 | 0.708      |
| Monocyte count (10$^{9}$/L)            | 0.5 ± 0.2 | 0.5 ± 0.2 | 0.6 ± 0.2 | 0.266      |
| Leukocyte count (10$^{9}$/L)           | 7.0 ± 1.6 | 6.8 ± 1.5 | 7.5 ± 2.0 | 0.269      |
| Neutrophil count (10$^{9}$/L)          | 4.3 ± 1.2 | 4.2 ± 1.2 | 4.4 ± 1.5 | 0.639      |
| Lymphocyte count (10$^{9}$/L)          | 1.9 ± 0.5 | 1.9 ± 0.5 | 2.1 ± 0.6 | 0.285      |
| Eosinophil count (10$^{9}$/L)          | 0.2 ± 0.3 | 0.2 ± 0.1 | 0.3 ± 0.4 | 0.329      |
| Basophil count (10$^{9}$/L)            | 0.04 ± 0.03 | 0.04 ± 0.03 | 0.05 ± 0.02 | 0.094      |
| CRP (mg/L)                             | 1.7 (0.5–3.5) | 1.5 (0.6–3.5) | 2.0 (0.5–4.8) | 0.984      |
| Basal serum tryptase (ng/mL)           | 5.5 (4.1–7.7); $N = 32$ | 5.5 (4.4–7.8); $N = 23$ | 6.1 (3.7–10.6) | 0.850      |
| Total IgE (IU/mL)                      | 113 (47–232); $N = 29$ | 94 (30–232); $N = 21$ | 143 (62–1101); $N = 8$ | 0.341      |
| Mean daily Temp. on the day when blood was drawn | 11.1 ± 7.6 | 12.4 ± 6.6 | 6.9 ± 9.4 | 0.077      |

Data are given as no. (%), mean ± SD, and median (IQR). If data was not obtained in all patients, patient numbers are displayed as “$N$” next to results. Statistical significance of differences between patient groups was calculated by Fisher’s Exact test, Mann-Whitney U test, and Independent-samples T test. No statistically significant $P$ values were found. 

**Abbreviations:** CG, cryoglobulin; ColdU, cold urticaria; CR(s), cold-induced reaction(s); CRP, C-reactive protein; CST, cold stimulation test; CSTT, critical stimulation time threshold; CTT, critical temperature threshold; IQR, interquartile range; $N$, number of patients; s, second; SD, standard deviation; UCT, Urticaria Control Test.