A point-of-care assay for alpha-1-acid glycoprotein as a diagnostic tool for rapid, mobile-based determination of inflammation

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Abstract

Background.—Inflammation is a key component of immune response to infections and pathogenesis of metabolic and cardiovascular diseases. Inflammatory biomarkers, including alpha-1-acid glycoprotein (AGP), are considered prognostic tools for predicting risk, monitoring response to therapy, and adjusting nutritional biomarkers for accurate interpretation. Serum is considered a primary source of biomarkers; urine and saliva are increasingly being explored and utilized as rapidly accessible, noninvasive biofluids requiring minimal sample processing and posing fewer biohazard risks.

Methods.—A lateral flow immunoassay was developed for an established mobile-based platform to quantify AGP in human serum, urine, and saliva. Assay performance was assessed with purified AGP in buffer, diluted human serum samples (n = 16) banked from a trial in people living with HIV, and saliva and urine (n = 15 each) from healthy participants. Reference methods were

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Author contributions
BMG and SM conceptualized and designed the study. BMG performed assay development, sample coordination, experimental work, and data analysis, and produced the initial manuscript. MG was the principal investigator on the parent study from which serum samples were obtained, coordinated regulatory approval, and served as a clinical mentor for the training award associated with this work. DE, MG, TR, JLF, and SM supervised the overall study. All authors reviewed and revised the manuscript and approved the final version.

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Competing interests statement: DE and SM have an equity interest in VitaMe Technologies (DBA VitaScan), which is commercializing similar diagnostic technology that is described herein. MG receives funding to his institution from Gilead Sciences for an investigator-initiated study unrelated to the current work. There are no further patents, products in development or marketed products to declare. BMG, JLF, TR: no conflicts of interest.
conventional clinical chemistry analyzer or commercial ELISA. Bootstrap analysis was used to train and validate sample calibration.

**Findings.**—The correlation between the assay and reference method for serum was 0.97 ($P < 0.001$). Mean (95% CI) best fit line slope was 1.0 (0.88, 1.15) and intercept was −0.003 (−0.08, 0.09). The correlation for urine was 0.93, and for saliva was 0.97 (both $P < 0.001$). The median CV for the LFIA for AGP in buffer was 13.2% and for all samples was 28.7%.

**Interpretation.**—The performance of the assay indicated potential use as a rapid, low sample volume input, and easy method to quantify AGP that can be licensed and adopted by commercial manufacturers for regulatory approvals and production. This has future applications for determining inflammatory status either alone or in conjunction with other inflammatory proteins such as C-reactive protein for prognostic, monitoring, or nutritional status applications, including large-scale country level surveys conducted by the DHS and those recommended by the WHO.

**PubMed indexing:**
Gannon; Glesby; Finkelstein; Raj; Erickson; Mehta

**Keywords**
lateral flow assay; ORM; orosomucoid; point-of-care; test strip

**1 Background**

Inflammation is central to immune response to infections as well as the pathogenesis of metabolic and cardiovascular diseases. Inflammatory biomarkers are additionally considered prognostic tools for predicting long-term chronic disease risk including cardiovascular disease and diabetes [1], and useful for monitoring response to therapy [2]. Inflammation is also associated with altered metabolism of both micro-and macronutrients. Adjusting micronutrient biomarkers for inflammation is required for accurate interpretation (Table 1) [3–6]. Commonly used biomarkers include C-reactive protein (CRP) and alpha-1-acid glycoprotein (AGP).

AGP, also known as orosomucoid, is a major plasma protein with diverse physiological roles including immune modulation, binding and transporting basic or neutral compounds including drugs, capillary barrier function maintenance, and metabolic regulation [7]. It is a positive acute phase protein that increases concentration in blood following various states such as infections, cancers, injuries, and obesity [7].

Concentrations of AGP have been associated with carotid plaque volume, men developing heart failure, increased risk of myocardial infarction and stroke, sepsis, and diabetes (Table 2) [1,8,9]. Further, AGP was shown to be a biomarker associated with metabolic syndrome compared to metabolically healthy overweight/obese women [10]. Urinary AGP excretion has been shown to be an independent predictor of cardiovascular mortality in patients with type 2 diabetes, which was also associated with elevated serum AGP concentrations [11]. Available and projected impact of determining inflammatory status to interpret nutritional
biomarkers and serve as diagnostic or prognostic indicators for various diseases or conditions is depicted (Figure 1).

AGP is typically used in conjunction with CRP to establish the time course and severity of infections that are known to impact biomarkers of micronutrient status [12]. The World Health Organization recommends measuring AGP along with CRP to adjust and interpret micronutrient biomarkers for vitamin A and iron in both research studies and large scale periodic population surveys conducted by its member states [3,5]. AGP also has been used as a component of the Prognostic Inflammatory Nutrition Index [13], which demonstrated prognostic power for postoperative complications, weight loss, and mortality following surgery for gastrointestinal neoplasia [14].

Serum AGP concentrations typically range from 0.4 – 1.2 mg/mL, but have been reported as high as approximately 3 mg/mL [15]. AGP rises more slowly and stays elevated longer than CRP; which can be used together to determine the stage and severity of inflammation, and has been suggested as a marker of long-term or chronic inflammation [6]. An AGP cutoff of 1.0 mg/mL has been used to adjust biomarkers of nutritional status for inflammation, however regression approaches have used concentrations of inflammatory biomarkers as continuous variables [16–18].

Determination of inflammatory biomarkers, including AGP, is typically done with ELISA or automated immunoturbidimetric assays using serum in a laboratory setting, which requires sample transportation and processing, skilled technicians, and laboratory equipment. However, populations at risk of either nutritional deficiency or chronic disease can have limited access to such monitoring or prognostic tools negatively affecting health outcomes, surveillance and program evaluation. Alternate quantification methods aiming to be easier and cheaper are being developed, but still require long sample preparation times [19]. Further, alternatives to blood sampling, including urine and saliva, are increasingly being explored as alternative biofluids with multiple collection and processing advantages [20]. Urinary and salivary inflammatory biomarkers, including AGP, have demonstrated applications including cancer, sepsis, and cardiovascular disease [20–22].

Lateral flow immunoassays (LFIAss) are typically paper-based assays that provide a qualitative or quantitative output. Advantages of LFIAss include the ASSURED criteria for diagnostics tests in resource limited environments as outlined by the World Health Organization: Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free, and Deliverable to end-users. Our research group has previously demonstrated quantification of biomarkers of iron, vitamin A, and CRP using a LFIA combined with a mobile-based strip reader and automated image processing [23]. In this study, we have developed a LFIA to quantify AGP with minimal sample preparation and a 15 minute assay time to complement and expand our diagnostic capabilities for nutritional and inflammatory biomarkers. We assessed the assay against purified AGP standard and human serum and validate the results against a reference immunoturbidimetric method.
2 Methods

2.1 Sample sources, participant characteristics, and ethical clearance

Serum samples used for assay validation came from a parent trial of Growth Hormone and/or Rosiglitazone for HIV-Associated Increased Abdominal Fat and Insulin Resistance (NCT00130286) [24]. Samples were identified as a convenient subset (n = 16 from 141) to include the widest range of AGP concentrations, balance between normal and elevated concentrations (> 1 mg/mL), and approximately evenly spaced throughout the range present in the overall sample.

Serum samples represented 16% females and a mean ± SD age of 46 ± 8 years.

Midstream urine and passive-drool saliva samples were collected from healthy adults in Ithaca, NY. Saliva and urine samples represented 60% females and a mean ± SD age of 30 ± 3 years. This work was approved by institutional review boards of Cornell University and Weill Cornell Medical College in New York, US.

2.2 Reagents, materials, and equipment

Antibodies included affinity purified goat anti-Human AGP (Lee Biosolutions, Maryland Heights, MO) and rabbit anti-goat IgG (Millipore Sigma, Burlington, MA). Gold nanoparticles were InnovaCoat 20OD 40 nm (Expedeon, Inc., San Diego, CA). Other reagents including purified human AGP standard, test strip membrane card (HF180), conjugate pad, and cellulose fiber pad from Millipore Sigma. Sample pads were obtained from mdi Membrane Technologies Inc. (Harrisburg, PA). A lateral flow reagent dispenser and syringe pump (Claremont BioSolutions, Upland, CA) were used to dispense antibodies.

2.3 Assay configuration and assembly

The AGP assay format is a sandwich immunoassay where the ratio of test (T) and control (C) line intensities (T/C) is proportional to the amount of AGP in the sample. The T/C has been shown to vary less with other experimental variables (e.g. temperature) and has been used to quantify different analytes using LFIA including fumonisins [25], vitamin B12 [26], and ferritin [27]. Assay development included selecting and trialing commercially available antibody combinations that allowed detection of AGP. Antibody concentrations were optimized to ensure dynamic range over the concentration range of interest. Components include a blood filtration membrane for sample input, a conjugate pad for holding gold nanoparticle (AuNP) anti-human-AGP conjugates, a nitrocellulose membrane with goat anti-human-AGP and rabbit anti-goat IgG antibodies, and a cellulose fiber wicking/absorbent pad. Antibody concentrations used were: AuNP-conjugated capture antibody: 0.1 mg/mL; test line antibody: 1.0 mg/mL; and control line antibody: 0.3 mg/mL. Antibodies were dispensed with a lateral flow reagent dispenser and dried at 37 °C for three hours. Anti-AGP antibodies were conjugated to AuNP per manufacturer instructions and diluted to achieve an optical density at 530 nm of 1 with conjugate buffer (2 mM borate buffer with 5% sucrose). The sample pad, conjugate pad, nitrocellulose membrane, and absorbent pad were assembled with 2mm overlap between components and test strips were cut to 4 mm width.
2.4 Sample preparation

Purified standards in buffer of different concentrations were prepared by diluting in 1 × PBS. Serum samples were stored at −80 °C or transported on dry ice prior to analysis. Serum samples were diluted 1,000-fold with 1 × PBS prior to analysis with the LFIA; undiluted concentrations are presented. Urine and saliva samples were centrifuged at 600 g for 10 minutes according to reference standard protocols, and supernatant was used undiluted for the LFIA.

2.5 Testing protocol

On each test strip, 7 μL of AuNP-anti-AGP conjugates was applied. For each test, 10 μL of sample was applied, followed by 60 μL of running buffer (1x TBS with 1% BSA, 1.5% Tween20, and 0.1% sodium azide) to initiate the test. After 15 minutes, the test strip was imaged, and subsequent image processing was performed as previously described [26]. Briefly, the image is converted to a greyscale 1D array, and local intensity maxima are determined to obtain the T/C ratio. All standards and samples were analyzed in triplicate.

2.6 Reference method

The AGP reference methods were conducted at the Cornell University Human Nutritional Chemistry Service Laboratory. Serum samples used a Dimension Xpand (Siemans, Erlangen, Germany) using a Randox AGP kit (Crumlin, United Kingdom) according to manufacturer’s instructions. Saliva and urine were analyzed with a commercial ELISA (Abcam, Cambridge, United Kingdom). ELISA values were corrected to reflect Dimension values using a linear transformation based on samples analyzed by both methods.

2.7 Data analysis

Mean T/C values for sample replicates were used for subsequent analysis. For standards in buffer, T/C values were compared directly to known concentration from diluted standard. For serum, saliva, and urine samples, T/C values were compared to AGP concentrations determined by the reference method with non-parametric bootstrap resampling analysis conducted in R (RStudio version 1.1.456, RStudio, Inc. Boston, MA) [28]. A linear model was fit to the bootstrap samples between T/C and log-transformed AGP concentration. Resampling was performed 1,000 times; means of estimates and standard errors are reported and compared to the reference standard. One serum sample appeared as an outlier, and analyses were conducted both with and without the outlier; results without the outlier are reported as estimates changed ≥10%. Three urine samples had CV > 15% by the reference method, and were not included in analysis.

3 Results

3.1 AGP-spiked buffer

Purified AGP in buffer demonstrated an increasing response in T/C between 0.25 and 6.0 μg/mL AGP (Figure 2). Negative control test strips did not display any T line signal while C line signal was still present (Figure 2B). A calibration curve was fit to the data of the form
T/C = 0.115*ln[AGP] + 0.182 with $R^2 = 0.984$. The median interassay CV% for AGP in buffer was 13.2%.

### 3.2 Serum from human participants

By the reference method, AGP concentration (mg/mL) of serum samples had a median (Q1, Q3) of 0.88 (0.50, 1.12) and a range of 0.19 – 1.49. Eight samples had AGP concentrations > 1 mg/mL.

The T/C ratio increased as serum AGP concentration increased with a best fit of the form $T/C = 0.16*ln[AGP] + 0.37$ with $R^2 = 0.95$ (Figure 3). With the outlier excluded, bootstrapping indicated the relationship between the LFIA and reference method with a mean (95% CI) slope of 1.0 (0.88, 1.15), intercept of 0.00 (−0.08, 0.09), and $R^2$ of 0.94 (0.93, 0.95) (Figure 3C).

Using a cutoff of 1 mg/mL, the assay was able to characterize serum AGP > 1 mg/mL with a sensitivity of 86% and specificity of 100%. Inclusion of the outlier reduced the sensitivity to 75%.

### 3.3 Urine from human participants

By the reference method, AGP concentrations (ng/mL) of urine samples had a median (Q1, Q3) of 0.39 (0.34, 0.48) and a range of 0.30 – 1.09. The T/C ratio corresponded to urinary AGP concentration with a best fit of the form $T/C = 0.48*ln[AGP] + 0.76$ with $R^2 = 0.86$ (Figure 4). The relationship between the LFIA and reference method with a mean (95% CI) slope of 0.69 (0.33, 0.95), intercept of 0.13 (0.04, 0.26), and $R^2$ of 0.86 (0.82, 0.88) (Figure 4C).

### 3.4 Saliva from human participants

By the reference method, AGP concentrations (ng/mL) of saliva samples had a median (Q1, Q3) of 0.48 (0.41, 0.69) and a range of 0.27 – 3.64. The T/C ratio corresponded to salivary AGP concentration with a best fit of the form $T/C = 0.50*ln[AGP] + 0.72$ with $R^2 = 0.88$ (Figure 5). The relationship between the LFIA and reference method with a mean (95% CI) slope of 0.76 (0.40, 1.02), intercept of 0.14 (0.00, 0.32), and $R^2$ of 0.95 (0.92, 0.96) (Figure 5C). The median interassay CV% across all samples was 28.7%.

### 4 Discussion

#### 4.1 Overall conclusion

We have demonstrated a LFIA that can rapidly quantify AGP concentrations from human serum, urine, and saliva using our mobile-based platform. We were able to establish an initial working range of standard in buffer and quantified samples with a calibration curve based on a bootstrap analysis unique to each sample type.

#### 4.2 Study strengths

Strengths of this study included the source of human samples, reference method, and established mobile-based platform used. The human serum samples used for assay validation
were selected from a pool of larger samples from a randomized controlled trial of people living with HIV to ensure that we captured the widest range of AGP concentrations possible. We used an automated Dimension Xpand Clinical Chemistry System as a reference method analyzed by a dedicated technician to ensure method reliability. Our mobile-based image capture and processing system has been validated to quantify a number of other analytes in both sandwich and competitive assay formats [23].

4.3 Study limitations

This study demonstrates the feasibility of the LFIA, and subsequent work will focus on translational aspects including assay optimization, automation, and evaluation. Serum samples were a convenient, non-random selection from a larger sample set; and measures of diagnostic accuracy and should be interpreted accordingly. Urine and saliva samples were sourced from apparently healthy adults; future work will need to additionally assess the assay in participants with diseases of interest. The demonstrated working concentration range of the assay includes mean values of controls and psoriatic [29] and cardiovascular [30] patients. The sample size was kept relatively small to evaluate a wide range of AGP concentrations while minimizing the number of assay lots and associated costs and variability for initial assay development. Strips were produced manually in an academic setting, and further optimization and automation with commercial equipment will reduce strip variability. Bootstrap analysis allowed us to develop standard curves specific for sample types as well as validate the LFIA results. Future studies will include more participants and established calibration curves with diagnostic test accuracy methodology.

4.4 Biomarker advantages

While determination of CRP is widely used in clinical practice and typically correlated with AGP, AGP has distinctive features that have established its use for nutritional assessment and merits further investigation in other domains. 1) A longer half-life than CRP (60–120 vs. 19 h, respectively) and therefore can better reflect long-term, chronic inflammation, 2) typically higher prevalence in population due to these kinetics, and 3) higher concentrations in serum, urine, and saliva, meaning undiluted saliva/urine concentrations are easily detectable by lateral flow assay permitting rapid quantification.

4.5 Assay advantages

The advantages of this assay primarily relate to the ability to conduct, analyze, and interpret results in a distributed fashion, especially benefitting those in more remote or resource limited settings. Lateral flow assays can be stored at ambient temperatures for prolonged periods with proper packaging [31]. This allows assays to be shipped and stored for use at the point-of-need without needing infrastructure for sample storage and shipment back to a central laboratory. Conventional chemistry analyzers can assay many samples in an automated, rapid fashion. However, the total assay time and resources required increases as patients are further from a laboratory. Addressing these barriers could increase availability of testing in populations or individuals that may otherwise not have access to facilities required for biomarker determination.
4.6 Applications and future directions

AGP is currently used to adjust or interpret nutritional biomarkers of iron and vitamin A status, which can complement established assays on our mobile based platform for retinol binding protein, ferritin, serum transferrin receptor, and C-reactive protein. It has also been specifically requested to include in future national nutrition surveys as a biomarker of chronic inflammation even in populations with low-infection burden to assess the impact of chronic inflammation on nutritional parameters such as iron deficiency [32]. AGP has demonstrated individual clinical diagnostic and prognostic applications for cardiovascular disease, diabetes, stroke, hospitalization, and growth, even when controlling for traditional risk factors (Table 2).

Future research directions will including 1) prospective diagnostic test accuracy studies to formally evaluate assay performance, 2) multiplexing with other nutritional or inflammatory assays to obtain multiple biomarkers in a cost and resource efficient manner, and 3) evaluate the test as a component of a clinical pathway to improve patient important outcomes.

Acknowledgements

The authors would like to thank the following: Victoria Simon, Cornell University Human Nutritional Chemistry Service Laboratory, for conducting reference tests. Lynn Johnson of the Cornell Statistical Consulting Unit for statistical consultation.

Funding. NIH/NCATS Grant # TL1-TR-002386 and NSF grant 1343058. Certain elements of the assay development were supported by NIH grant R03 EB 023190. The parent trial supplying banked samples was supported by NIH (R01 DK065515, K24 AI078884, UL1 RR024996, M01RR 00047, M01 RR 00645-27, UL1 TR000040-07, and P30 DK026687). The funders had norole in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Abbreviations used:

- AGP: alpha-1-acid glycoprotein
- BRINDA: Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia
- CRP: C-reactive protein
- INSPIRE: Inflammation and Nutritional Science for Program/Policies and Interpretation of Research Evidence
- LFIA: lateral flow immunoassay
- sTfR: serum transferrin receptor
- T/C: ratio of test and control line intensities

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FIGURE 1.
Available and projected impact of determining inflammatory status to interpret nutritional biomarkers and serve as diagnostic or prognostic indicators for various diseases or conditions.
FIGURE 2.
(A) Representative images of test strips demonstrating varying T/C intensities at different concentrations of purified human AGP. (B) Representative images of negative control test strips demonstrating C line signal, but no T line signal. (C) Calibration curve of T/C obtained from the lateral flow assay against known concentrations of purified human AGP. Data are mean ± SEM.
FIGURE 3.
(A) Representative images of test strips demonstrating varying T/C intensities at different concentrations of AGP in serum determined by the Dimension Xpand reference method. (B) Calibration curve of T/C obtained from the lateral flow assay against known AGP concentrations in human serum. (C) Comparison of AGP concentration estimates obtained from the LIFA and bootstrap analysis against AGP concentrations determined by the Dimension Xpand reference method. Open circle is outlier excluded from analysis. Dashed line is best fit line. Data are mean ± SEM. Samples were diluted for analysis for both reference standard and the LFIA; reported data represents undiluted concentrations.
FIGURE 4.
(A) Representative images of test strips demonstrating varying T/C intensities at different concentrations of AGP in urine determined by the commercial ELISA reference method. (B) Calibration curve of T/C obtained from the lateral flow assay against known AGP concentrations in human urine. (C) Comparison of AGP concentration estimates obtained from the LIFA and bootstrap analysis against AGP concentrations determined by the adjusted ELISA reference method. Dashed line is best fit line. Data are mean ± SEM.
FIGURE 5.
(A) Representative images of test strips demonstrating varying T/C intensities at different concentrations of AGP in saliva determined by the commercial ELISA reference method.
(B) Calibration curve of T/C obtained from the lateral flow assay against known AGP concentrations in human saliva. (C) Comparison of AGP concentration estimates obtained from the LIFA and bootstrap analysis against AGP concentrations determined by the adjusted ELISA reference method. Dashed line is best fit line. Data are mean ± SEM.
Table 1

Selected evidence, recommendations, and use of inflammatory biomarkers, including AGP, for nutritional biomarkers

| Reference                                      | Organization                                      | Type                               | Biomarker                  | Findings/Recommendation/Usage                                                                                                                                                                                                 |
|------------------------------------------------|--------------------------------------------------|------------------------------------|----------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| (Thurnham, 2003) [12]                          |                                                  | Meta-analysis                      | Retinol                    | Correction factors established for elevated CRP and/or AGP. Recommended measurement of both due to highest magnitude of change when both are elevated                                                                                       |
| (Thurnham, 2010) [33]                          |                                                  |                                    | Ferritin                   | " "                                                                                                                                                                                                                      |
| (WHO, 2011) [3]                                | World Health Organization                        | Recommendation                     | Retinol                    | Knowledge of infection burden is critical for accurate interpretation                                                                                                                                           |
| (WHO, 2011) [5]                                | " "                                             | Recommendation                     | Ferritin                   | Include CRP and AGP                                                                                               |
| (WHO/CDC, 2007) [6]                            | World Health Organization, Centers for Disease Control | Technical consultation report   | Iron status                | Include at least one acute phase protein, including CRP and AGP as first choices. AGP may be a better indicator of the presence of chronic, sub-clinical inflammation.                      |
| (Raiten, 2015) [4]                             | INSPIRE                                          | Working group evidence review and recommendations | Nutritional biomarkers    | One or more biomarkers of inflammation should be measured if population has known/suspected inflammatory burden. The addition of AGP as a measure of longer-term inflammation is probably useful in most settings and should be measured in addition to CRP. |
| (Larson, 2018) [34]                            | BRINDA                                           | Application to nationally-representative surveys | Retinol, retinol binding protein | CRP and AGP included in adjustment strategies                                                                                                                      |
| (Namaste, 2017) [35]                           | " "                                             |                                    | Ferritin                   | CRP and AGP included in adjustment strategies                                                                                                                      |
| (Rohner, 2017) [18]                            | " "                                             |                                    | sTfR                       | AGP used alone due to stronger relationship with sTfR                                                                                                           |
| (Mei, 2017) [17]                               | " "                                             |                                    | Total body iron (ferritin and sTfR) | Ferritin adjusted with CRP and AGP and sTfR adjusted with AGP only                                                                                               |

AGP: alpha-1-acid glycoprotein, BRINDA: Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia, CRP: C-reactive protein, INSPIRE: Inflammation and Nutritional Science for Program/Policies and Interpretation of Research Evidence.

sTfR: serum transferrin receptor.
## Table 2

| Reference | Application | Population | Study | Outcome | Results |
|-----------|-------------|------------|-------|---------|---------|
| (Schmidt, 1999) [36] | Prognosis | US adults, n = 12,330 | Prospective cohort, mean follow-up: 7 years | Incidence of diabetes | OR (95% CI) 7.9 (2.6, 23.7) for above vs. below median AGP. |
| (Engstrom, 2009) [37] | “” | Swedish men without history of MI or stroke, n = 6,071 | Prospective cohort, mean follow-up: 22 years | Incidence of hospitalization due to heart failure | Baseline AGP higher in those developing heart failure. HR (95% CI): 1.5 (0.9, 2.4) for AGP Q4 vs. Q1, and 2.6 (1.6, 4.1) for ≥ 3 acute phase proteins, including AGP, elevated. |
| (Berntsson, 2016) [8] | “” | Swedish adults, n = 4,285 | Prospective cohort, median follow-up: 17.7 years | Incidence of stroke | HR (95% CI): 1.48 (1.02, 2.16) for AGP T3 vs. T1. 2.07 (1.38, 3.11) for AGP T3 vs. T1 + T2. |
| (Lee, 2016) [38] | “” | Nepalese children, n = 249 | Longitudinal cohort, follow-up 1 year. | Universal Nonverbal Intelligence Test | AGP was negatively associated with the Universal Nonverbal Intelligence Test after one year of follow-up. |
| (Bao, 2018) [1] | “” | Swedish adults, n = 4,322 | Prospective cohort, mean follow-up: 17–18 years | CVD incidence | Per 1 SD of AGP: HR (95% CI): 1.19 (1.11, 1.27) * HR (95% CI): 1.09 (1.01, 1.17) ** |
| “” | “” | “” | “” | Diabetes incidence | Per 1 SD of AGP: HR (95% CI): 1.29 (1.20, 1.38) * HR (95% CI): 1.12 (1.03, 1.21) ** |
| (Xiao, 2015) [9] | Diagnosis | Chinese adults with suspected sepsis, n = 277. | Cross-sectional diagnostic test accuracy | Sepsis | AGP demonstrated ability to differentiate systemic inflammatory response syndrome from sepsis and the degree of sepsis. |
| (Ley, 2016) [39] | Response prediction | French children with Crohn’s disease, n = 107. | Longitudinal cohort, followed up at least 2 years. | Growth velocity | Growth velocity negatively associated with AGP |
| (Shah, 2017) [40] | “” | US adults with relapsed/refractory multiple myeloma or plasma cell leukemia, n = 85 | Overall clinical response to Filanesib | Only patients with AGP < 110 mg/dL responded to treatment with Filanesib |

CVD: Cardiovascular disease; HR: Hazard ratio; OR: Odds ratio; T: tertile; Q: quartile.

* Multivariate model adjusting for age and sex.

** Multivariate model adjusting for age, sex, and other risk factors for outcome of interest.