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Journal Name:	**Advances in Research**
Manuscript Number:	**Ms_AIR_24734**
Title of the Manuscript:	**Biosensor strategies to detect serum glycobiomarkers**
Type of the Article	**Review paper**

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PART 1: Review Comments

	Reviewer's comment	**Author's comment** *(if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)*
Compulsory REVISION comments	Numerous inaccurate and confusing statements have been found in the text concerning glycosylation process itself and application of lectins as glycobiological tools that are listed below. 1. Lanes 60-62 Although some glycosidases are engaged in N-glycan maturation, the main enzymes remain glycosyltransferases; the statement " glycosyltransferases and glycosidases" would be much more appropriate 2. Lane 59 "human plasma proteins are glycosylated, in addition to glycosylated proteins secreted..." Plasma proteins are secreted and mostly glycosylated, so what was the idea of this distinction? It doesn't make sens. 3. Lane 74 and following N-glycan is firstly synthesized as membrane anchored lipid-linked precursor, dolichyl phosphate + 14 monosaccharide units (GlcNAc2Man9Glc3) and in this form transferred to the glycosylation sequon of the nascending protein 4. Lane 76	

	" … the trimannosyl … precursor is cleaved …" NO! The 14-residue glycan is cleaved up to pentasaccharide core (trimannosyl) – this one is no longer cleaved, but elongated to form mature N-glycan 5. Lanes 79-81 In mammals, both hybrid and high-mannose type N-glycans are extremely rare: this is yeast/plant/invertebrate type of glycosylation 6. Lane 82 and following Mature mammal N-glycans never contain glucose! The triglucosyl tail is a signal for calnexin-calreticulin chaperon system and is removed when protein is correctly folded 7. Part 4: Glycobiomarkers The authors fairly confuse two distinct features: protein synthesis and glycosylation. When the level/concentration of a glycoprotein like haptoglobin, alpha fetoprotein or PSA is measured, for example with double antibody-sandwich ELISA (or any other technique directed to the polypeptide chain) – we cannot denominate it as glyco-biomarker, even when the measured protein contains carbohydrate moiety. The measured feature is related to protein synthesis, not glycosylation. When the measurement concerns directly sugars (for example "core-fucosylated AFP", lane 155,) – the application of "glycobiomarker" name is appropriate, as presence, absence or content of	

	carbohydrate is in focus. For this type of assay, carbohydrate-recognizing ligand is necessary (i.e. lectin or carbohydrate specific antibody) 8. Part 6. Lectins as biorecognition elements Lectins are ligands of choice in glycobiological studies, as carbohydrate-specific antibodies are hard to be obtained. This is not surprising, as the immune response is based on self/non-self distinction, and in general glycan structures are common for all mammals, so they are mostly recognized as "self". Thus, the panel of lectins used as tools in glycobiology is really wide. From these, the authors focus their interest on ConA, SNA, PNA and *C. mollis*. Except of SNA, these lectins are characterized with wide carbohydrate specificity, and this strongly limits their application. The more frequent problem in lectin-based glycobiology research is to find and apply the lectin specific enough to ensure the detection of carbohydrate structure of interest (and not the glycan similar and more abundant). This is the main limit in lectin-based research. Unfortunately, the authors do not discuss lectin cross-reactivity and its impact on the interpretation of experimental data. In my opinion, also some explanation, why the authors focus their interests on the lectins of such a wide specificity and what are their expected benefits, would be appreciable in this article.	

Minor REVISION comments	1. Resolution of figures is too low – they are hardly readable 2. In Figure 1 antennary fucosylation of N-glycans, one of the most common structural features of these carbohydrates, is completely omitted. On the other hand, the presence of core fucose residue seems to be obligatory, and that is not true. 3. In Figure 2 "R" description: it cannot be Ser/Thr hydroxyl group, as in O-glycans N-acethyl-glucosamine residue is never directly attached to the polypeptide backbone. "O-glycan core (or extended O-glycan core)" would be correct 4. Section2 – Glycosylation: This section presents well established general information on the glycosylation pathway, but the citations lead the reader to very detailed experimental studies, instead of general review or textbook. I strongly recommend "Essentials of Glycobilogy 2nd edition" for example (free access in the internet). This will also help to avoid wrong explanations of glycosylation patway (see major remarks) 5. lane 340: the text concerns T-antigen, while the citation [91] refers to Cramoll lectin induced mitogenic response – once again the citation is questionable. 6. Figure 5: the 4th example with double lectin detection requires multivalency of carbohydrates (rathe a common feature in glycosylation). This is shown in the figure, but should be also explained	

	in the legend and the text. Are these lectins identical or different? This determines different strategies of the measurement. 7. English language needs serious revision – there are numerous spelling errors (research instead of researchers, easily instead of ease or easy and so on, confused singular and plural, simple past and past participle, wrong prepositions)	

Optional/General comments	Glycobiology is a rapidly developing area of research . Contribution of carbohydrates in numerous cell-cell or protein-cell receptor interactions is unquestionable today. It also becomes clear that such cross-talk is not only crucial for proper function of an organism, but also its impairments are engaged in etiology of different diseases. In the latter case the possibility to detect altered carbohydrate structures may be of enormous diagnostic importance. At the same time the level of knowledge in this field among both medicals and biologists remains insufficient. For this reason every attempt to improve reception of these problems is valuable, thus the article under review concerns important issues. The main problem in glycobiological research is associated with enormous structural diversity of glycans that generates/remains serious technical/methodological challenge. Thus the review focused on one of the modern possibilities of sensitive measurements in this area should be regarded as relevant. Unfortunately the authors of the current article failed to avoid some serious errors in their description of glycosylation pathway, confirming the opinion that glycobiology is still an area not familiar enough even to the researchers. These issues will be addressed in detail in the "compulsory revision" section. Concluding, although in the article under review	

	numerous shortcomings and inaccuracies are present that require serious major revision, in my opinion after such re-writing it may be really valuable piece of information, explaining modern possibilities of research in the important and dynamic field of glycobiology.	

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